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POTATO BLACKLEG WITH SPECIAL REFERENCE TO THE ETIOLOGICAL AGENT[1]

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Introduction

The objects of the investigations presented herewith were several, but only those outstanding should be mentioned at this point. First of all, a determined effort was made to discover the relationships, one to another, of the several "species" of bacteria recorded as being the cause of the blackleg disease of potatoes. To this end a thorough comparative study of the morphology, cultural features, and physiology of some 12 strains of the blackleg bacillus was made. The cultures employed represented strains of the organism from regions widely separated geographically. Among them were the 4 "species" originally described as being the cause of the disease in question. In the prosecution of the problems arising the writer was led into a quantitative study of carbohydrate utilization by strains of the blackleg bacillus and other microorganisms. The work done in this connection constitutes an important phase of the investigations carried out. By way of extending the usefulness of the paper, the writer presents a rather full and complete diagnosis of the disease, with a discussion of the economic aspects, also a revised description of the potato blackleg parasite.

[1]An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment for the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

Historical

Frank ('97, '99) was the first to publish accurate and detailed descriptions of the potato blackleg disease. One may be assured he was discussing the potato disease which goes by the name of "blackleg" and which is so common and well known in many parts of North America. He reported the cause of the disease to be a bacterium which he briefly described and named *Micrococcus phytophthorus*. Micrococci have been frequently met with in isolation plates made from potato stems and tubers affected with the blackleg disease. Many other workers have reported similar experiences, but no one has presented adequate proof that any one of the species of *Micrococcus* found present under such circumstances is pathogenic to the potato. Until such time as a species of *Micrococcus* conforming to Frank's description shall be proved capable of infecting potatoes and causing the blackleg disease, *Micrococcus phytophthorus* Frank should be considered a *nomen nudum*.

About 20 years earlier Hallier ('78) described an infectious wet rot of the potato and he discussed at considerable length the association of certain bacteria ("Vibrionen," "Bakterien," "Micrococcus") and a fungus (*Peronospora infestans* Casp.) in the affected potatoes. One cannot determine from the text which of the organisms is of primary significance, but as a mere matter of historic interest it may be noted that this writer contended that the wet rot described by him was caused by "die Produkte der Plastiden der *Peronospora infestans* Casp." While it appears unlikely that the wet rot described by Hallier is in any way related to the rot due to the blackleg germ, it is nevertheless interesting to note the mention at this early date of a potato tuber rot in which bacteria were found present.

D'Arbois de Jubainville and Vesque ('78), nearly 45 years ago, showed that they were familiar with a potato tuber rot ("pourriture cellulaire") occurring in the soil, but the bacteria were not identified as the cause; rather, the disease was thought by them to be due to an excess of nutrients and water.

A year later Reinke and Berthold ('79), as a result of studies of potato decay due to fungi, concluded that a wet rot of potato tubers existed which was not due to fungi. Furthermore, they demonstrated the association of bacteria with the wet rot described, and they also proved the infectious nature of the rot.

Burrill ('90, '93) published brief notes on a bacterial disease of potatoes, but as nearly as can be determined, the disease which he studied was the brown rot of potato, later most thoroughly investigated by E. F. Smith.

A bacterial malady of the potato was described by Prillieux and Delacroix ('90) in France, and what they took to be the causal organism was designated *Bacillus caulivorus*. The potato disease referred to by Prillieux and Delacroix was described as one which affects the stem, beginning at the bottom and working upwards. To this extent, at any rate, it is comparable to the one under consideration. No description of the cultural characteristics of the organism was presented in their paper and no statement was made concerning the method of isolation by which the organism was obtained. A few years later Prillieux ('95) briefly mentioned certain of its characteristics, stating that it developed a green coloration in some media. Laurent ('99) stated that *B. caulivorus* was probably the common saprophyte *B. fluoresence liquefaciens* Flügge. Later, Delacroix concluded, according to Prunet, that *B. caulivorus* was most probably *B. fluorescence liquefaciens* Flügge, which he thought became parasitic under certain conditions. A more complete review of the work referred to just above will be found in Prunet's ('02) paper.

One of the early contributions to our knowledge of bacterial diseases of plants in general and of the potato in particular is that of Kramer ('91), who describes quite fully a wet rotting of potatoes. He obtained pure cultures of the supposed etiological agent which he described as a spore-bearing, rod-shaped bacterium.

The potato disease described by Tyron ('94, '99) as occurring in Queensland, Australia, is probably not the blackleg disease. Rather according to Tyron's statements, it is very similar to, if not the same as, E. F. Smith's brown rot disease (see especially Tyron, '99, "Biography," p. 62, and footnote p. 63; also Smith, '14, p. 208).

Smith's first publication on the brown rot of *Solanaceae* caused by *Pseudomonas (Bacterium) solanacearum* E. F. S. appeared in 1896. From the outset there was little or no confusion of this disease with the one under discussion. This paper by Smith is one of the first thoroughgoing and accurate descriptions of a bacterial disease of the potato. Recalling that his work was

done at an early date, and at a time when foreign bacteriologists of high standing opposed the idea that bacteria could be the primary cause of disease in plants, the contribution is all the more significant.

Wehmer ('98) published extensively the results of his investigations of potato diseases. In Part 3 he describes a bacterial rot of the tuber, but he definitely takes the attitude that the bacteria found associated with the rot were not the primary etiological agents. His attitude may be taken as representative of the belief of most German botanists and bacteriologists of that time.

There occurred in the vicinity of St. Petersburg, Russia, in 1898, a potato bacteriosis which was described by Iwanoff ('99). The causal organism was described as a short, oval-cylindrical, active rod found (in the tissues) to measure 1.5μ × 0.5μ. The disease was one which affected the leaves and stems, early symptoms being manifest in the leaves. Later, the stems were affected and showed symptoms of the disease. Brown lesions appeared externally on the stems, the pith was attacked and destroyed, and the stems wilted and died. Starch was not destroyed. Iwanoff contended that the disease he described was similar to that described by Smith ('96) as due to *B. solanacearum*. It appears to the writer that he was mistaken in the view presented, and from our limited knowledge of the situation it seems more likely that the potato disease prevalent in Russia near St. Petersburg in 1898 was the blackleg disease.

Jensen ('00) investigated a bacterial disease of the potato which he referred to as "blackleg". As a result of microscopic investigations of potato plants affected with "blackleg" he concluded that the disease was caused by bacteria ("Mikrokokken").

What Smith ('14) refers to as "The French Disease" was first described by Delacroix ('01) in a succession of short papers appearing in 1901. Delacroix first stated that the potato malady due to *B. solanacearum* E. F. Smith was prevalent in France. Later, he concluded that the disease referred to above, the one for which he suggested the name "brunissure," was caused by a bacterium new to science which he named *Bacillus solanicola*. Smith ('14), however, claimed that, "Here again, it is uncertain whether we have to do with *Bacillus phytophthorus, Bacterium solanacearum,* or some third organism. The writer obtained a

culture of *B. solanicola* from Prof. L. R. Jones, to whom it was given in Paris by Delacroix, but either it never possessed any pathogenic properties, which is quite probable, or else had lost them by cultivation." The same investigator made the following statement, after having had opportunity to examine material collected and preserved by Delacroix to illustrate the disease: "It represents a fungous disease of the potato."

Part III of van Hall's ('02) doctoral dissertation ("The stem rot or blackleggedness of potato stems caused by *Bacillus atrosepticus* nov. sp.") gives a fairly complete description of the disease under discussion, together with a diagnosis of the etiological agent sufficiently detailed and complete to enable one to classify the parasite. For reasons which appear below, the writer is of the opinion that the potato disease commonly referred to throughout the United States and many parts of Europe as "blackleg" is one and the same thing as that described by van Hall. The etiological agent in all cases is probably identical and, as far as is known, should be referred to van Hall's *Bacillus atrosepticus.*

At a slightly earlier date Appel ('02) published a short paper ("Contribution to our knowledge of the bacterial rotting of potatoes"), in which he described effects of the rot, also experiments on the pathogenicity of the disease and stated that the systematic position of the causal agent of the potato rot, as well as its practical importance, would be given in a forthcoming article. About a month later another short paper was published by the same investigator (Appel, '02a), entitled "The cause of the 'Blackleggedness' of the potato." In this article he takes exception to Frank's binomial in the following words: "Wenn nun auch der Frank'sche Name *Micrococcus phytophthorus* auf den Bacillus nicht anwendbar ist, so glaube ich doch aus praktischen Grunden den Speciesnamen beibehalten zu sollen, um so mehr als es sich herausstellte, dass eine ganze Reihe von Pflanzen in charakteristischer Weise angegriffen wird. Ich nenne daher den von mir isolirten Bacillus, welcher Schwarzbeinigkeit und Knollenfaule bei den Kartoffeln hervorruft: *Bacillus phytophthorus* Appel." Nowhere in this paper, however, is there to be found a description of the morphological and cultural characters (other than those implied by the name) of the etiological agent which he had isolated. The binomial given by Appel in this publication must therefore be regarded as a *nomen nudum.*

In 1903 Appel ('03) published a complete account of the potato blackleg disease as it occurs in Germany, together with a description of the causal organism. The same investigator (Appel, '05, '06) contributed substantial additions to our knowledge of this disease.

Butler ('03) mentions a bacterial disease which he thought was possibly the same as that described in the United States by E. F. Smith under the name of brown rot.

Jones ('05), in an account of disease resistance of potatoes, presented a description of the blackleg disease and accompanied it with observations upon its occurrence in Europe. He reported that it was found in Holland, Belgium, Germany, France, and England. The first authentic record of the occurrence of potato blackleg in the United States is that of Jones ('07) who found it in 1906 on a farm in Vermont. The seed used in planting the field came from Maine. He states also: "Vague reports have frequently come to the Station in previous years as to troubles of this class . . ." This author (Jones, '07) "passed almost directly from German fields where it was prevalent, to English fields and found the malady equally common and identical in appearance with that on the continent." This statement is of especial significance in connection with the report of Johnson ('07) who claims to have gathered substantial evidence of the existence of the potato blackleg disease in Ireland. (Compare also with the report made by Pethybridge and Murphy in 1910).

Harrison ('07) published a very complete account of a potato rot occurring in eastern Canada. The symptoms of the disease described by him were, he states, quite similar to the "Schwarzbeinigkeit" of van Hall and Appel. While he attributes the disease to a bacterial species (*Bacillus solanisaprus*) new to science, the writer has reason to believe (see below) that the causal agent described by him is identical with *Bacillus atrosepticus* van Hall. Morse ('07) found potatoes affected with blackleg in Maine in 1907. In 1909 he (Morse, '09, '11) published an account of the nature of the disease, its distribution, economic importance, and its control in Maine.

Smith ('10), however, was the first American to publish a full account of the blackleg parasite. His description is based on studies made with a subculture of Appel's species which he obtained from Aderhold in Berlin, presumably a transfer from

Appel's original culture. Smith states in this article that he regards *Bacillus solanisaprus* Harrison as closely related to, but not identical with, *B. phytophthorus* Appel.

About the same time Pethybridge and Murphy ('10) published a note on a bacterial disease of the potato plant, attributing it to a new species of bacterium for which they proposed the name "*Bacillus melanogenes.*" In 1911 these investigators published a full account of the malady referred to above, together with a complete diagnosis of the etiological agent. The potato disease described by them was probably none other than the "blackleg," and there is very little, if any, doubt in the writer's mind that their *Bacillus melanogenes* should be referred to *Bacillus atrosepticus* van Hall.

Murphy ('16) gives an account of a blackleg disease of potatoes occurring in Canada, attributing the disease to *Bacillus melanogenes* Peth. & Murphy.

From a series of observations made in 1916, Morse ('17) reports having seen potato blackleg in certain of our western states, though he found it to differ in certain respects from that familiar to him in Maine. This paper is an important contribution to the literature of the subject. Of particular significance are his comparative studies of the causal organisms. The writer is in accord with Morse's conclusion that the strains studied (including cultures "received under the names '*Bacillus atrosepticus* van Hall,' '*B. solanisaprus* Harrison,' and '*B. melanogenes* Peth. & Murphy'," as well as 3 isolated from blackleg material in Maine) are identical. However, since Morse was unable to procure a trustworthy culture of *B. phytophthorus* Appel he presents no data which "bear on the relationship between the organism originally described by Appel as *B. phytophthorus* and the other strains of blackleg bacteria." So in deference to the opinion of Smith ('10) that *B. phytophthorus* Appel is not identical with *B. solanisaprus* Har., Morse was forced to exclude it from *B. atrosepticus* van Hall. This is most regrettable, since Morse shows that small differences in the physiology of bacteria are not sufficient for the establishment of new species. His work shows abundant evidence of having been carefully done. Unfortunately, there seems to be no record of the comparative studies upon which Smith ('10) bases his opinion.

Paine ('17) referred to the cause of the potato blackleg studied by him in England as *Bacillus atrosepticus* van Hall and ex-

pressed the opinion that the species described by Appel, Harrison, and Pethybridge and Murphy should be referred to van Hall's *Bacillus atrosepticus*. The argument advanced by this investigator is most logical, but attention is called to the fact that he did not carry out comparative studies using the several "species" in question.

Rosenbaum and Ramsey ('18) contributed the results of some studies upon the influence of temperature and precipitation on the blackleg of potato.

Ramsey's ('19) studies of the viability of the potato blackleg organism led him to believe that the pathogen does not live in the tubers left in the field over winter. He expressed the further conclusion that unless the potato seed were infected at planting time there was little chance that the uninjured plants would contract the disease.

Artschwager's ('20) researches upon the pathological anatomy of potato blackleg form a new contribution to our general knowledge of this disease. He studied only blackleg plants grown in the arid regions of western Colorado. The affected plants examined by him were found to show an increase in strongly lignified vascular tissue and a transformation of part or most of the parenchyma cells of the cortex and pith into sclerids. This investigator also discovered that protein crystals occurred in great abundance in all organs of plants affected with the blackleg disease, especially in the leaves, while under normal conditions protein crystals have been observed only in the peripheral cell layers of the cortex of the potato tubers.

Jennison's ('21) abstract, "*Bacillus atrosepticus* van Hall, the cause of the blackleg disease of Irish potatoes" was based on the investigations carried out in large part in 1916 and 1917, here reported upon in detail. While the group number as published by the writer at that time differs in respect to the last 3 digits from that of Morse ('17), it agrees throughout with that assigned by Shapovalov and Edson ('21). The last-named investigators contributed one of the most important of the recent accounts of the disease under discussion. They showed that the potato blackleg disease prevailed in the irrigated districts of the West, and that the causal agent was a *Bacillus*, which they concluded was "identical with *Bacillus phytophthorus* Appel in all the essential characteristics considered in determining bacterial spe-

cies." A detailed description of the causal organism isolated by them was given.

I. Diagnosis of the Disease

As indicated in the preceding section, the blackleg disease of potatoes is a common and destructive bacterial disease of many varieties of *Solanum tuberosum*. The common name "blackleg" has been widely used in the United States and Canada, since it was first introduced by Jones in 1905. The term "blackleg" is a rather free translation of "Schwarzbeinigkeit," under which name the disease is known in Europe. The writer feels that blackstem and rot would be more suitable for American use but sees no point in suggesting that a change be made at this time, because (1) the old name has become well established and (2) because of its adoption by the American Phytopathological Society's Committee on Common Names for Plant Diseases. It may be pointed out, however, that the writer has observed that the term "blackleg" leads some farmers and others, especially in the West, to think that it may be related to the blackleg disease of cattle. More particularly is this the case when they learn that both are caused by bacteria.

Type—Potato blackleg is primarily a parenchyma necrosis, the cortical and pith tissues of both stem and tuber being almost exclusively involved. Occasionally, the vascular elements are found to be invaded by the parasite and sometimes they are somewhat browned.

Signs of blackleg in the field.—Considerable importance attaches to general manifestations of the disease as it appears in the field, since upon such manifestations depends the important practice of roguing, advocated below. Not infrequently "skips" or "missed hills" are due to this disease, for under favorable circumstances the young sprouts are destroyed by the parasite before they appear above ground; also the seed piece may be destroyed before it sprouts. When the disease progresses rapidly the plants wilt and become blackened to a considerable height. Such plants are quickly prostrated and soon die (pl. 1, fig. 3).

Secondary symptoms of the disease are likely to be the first to appear. Undersized vines with more or less yellowed and rolled leaves should be regarded with suspicion and more closely examined for the presence of black lesions upon the stem, both above

and below ground. There is, furthermore, a noticeable tendency for the upper leaves in particular to manifest a somewhat xerophytic texture and a starkness of growth-habit, the total effect being to give the tops of affected plants a narrowed or contracted appearance. Besides being light-colored, the upper leaves are often rendered more conspicuous by the presence of a metallic luster. Plants affected with blackleg, as a rule, offer little resistance to removal from the soil, and not infrequently break off when one attempts to pull them. If upon removal from the earth dark-colored cortical lesions are found on the underground portions of the stem, there is little doubt but that the plant is attacked by the blackleg organism. When upon splitting the stem longitudinally the pith is found blackened and more or less disintegrated the case is quite clear (pl. 1, figs. 2 and 4). Further confirmation is afforded if the seed piece has disintegrated. Aerial tuber development due to attack by the blackleg parasite is not commonly seen. The production of aerial tubers does take place when practically all the tuber-bearing stolons on well-developed plants are destroyed by the pathogen, thus precluding normal tuber development. *Rhizoctonia* more frequently causes such abnormalities of tuber development, and the underground lesions due to this fungus may be confused with those due to *Bacillus atrosepticus*. Certain characteristics of the *Fusarium* wilt disease may also be confused with the yellowing and wilting of potato vines caused by the blackleg parasite.

As a rule, plants affected with blackleg occur scattered promiscuously over the field. The occurrence and spread of the disease appear to bear a definite relation to environmental conditions, and the number of diseased plants is likely to be greater during a cold wet spring than in a warm dry one. Not infrequently there are more diseased plants in the low, poorly drained spots in a field.

"Vine" Symptoms.—The primary symptoms of the disease as they occur on the potato vines are most striking. These appear first, as a rule, on the lower parts of the stem both below and above ground, and are characterized by dark brown to black lesions or cankers, hence the origin of the German name "Schwarzbeinigkeit," or "blackleggedness." The first cankers are likely to develop below ground and not infrequently have their beginning at the lowermost part of the stem. Under favorable circumstances the disease works rapidly upward. On young succulent

stalks a lateral development of a lesion promptly results in a girdling of the stem, causing death. Under natural conditions the stem is seldom, if ever, first infected above ground. There is little tendency for the pathogen to migrate downward, even when stems are artificially inoculated at a point above ground. This fact is strongly emphasized by Smith's ('20) figures 193 and 199. The strong tendency on the part of the parasite to migrate upward along the main stem is shown in pl. 1, fig. 1. This photograph illustrates a rather striking, though not uncommon, case. Upon closer examination it will be found that the bacteria are largely confined to the parenchymatous tissues of the stem to the cortex and the pith. As it works upward in the pith this colorless parenchyma also becomes blackened and necrotic (pl. 1, fig. 4), easily observed by splitting the stem longitudinally. In this manifestation we have one of the most striking as well as reliable diagnostic features. The pith finally disintegrates, leaving the stalk more or less hollow. Frequently worms will be found feeding among the dead pith cells.

Lateral spread in large mature stems is very slight. Even though the stalk is severely diseased the plant may persist and mature a crop of tubers (pl. 2, fig. 3). Under favorable circumstances the infective agent moves through the stolons to invade the tubers. Sometimes the latter organs are infected at other points, invasion probably taking place for the most part through the lenticels, as has been experimentally demonstrated by Smith ('20). Diseased tubers frequently rot in the ground, the entrance of saprophytes bringing about the profound, slimy, putrid, soft-rot frequently observed. Death of the roots is secondary and follows a killing of the tops.

Tuber symptoms.—As implied above, stem-end infection of the tubers is most frequent under ordinary circumstances. As a rule, decay begins at the point of attachment of the stolon (pl. 2, figs. 3 and 4). Often, however, comparatively little decay is visible. Experiments carried out by the writer showed that the pathogen may be securely lodged in the tuber at the juncture with the rhizome, even though there are no signs of rot. He has also determined on many occasions that the blackleg parasite is the cause of more or less discoloration in the tissues associated with the vascular elements in the stem-end of the tubers. Such signs of the disease may readily be mistaken for symptoms of the *Fusarium* and *Verticillium* wilt diseases. In-

fection of the eye or bud end is less commonly found. A stem-end rotting of the Russet Burbank (Netted Gem) variety, due to *Bacillus atrosepticus,* is well known in many of the irrigated sections of the Northwest. It is most frequently seen at digging time, though it may be found earlier, especially if the latter part of the growing season is cool and rainy. Affected tubers are often found having pointed ends, a characteristic of some value, even though not proof-positive, since signs of the rot are more or less masked in freshly dug potatoes. Shrinkage of the affected tissues follows exposure of the tubers to drier surroundings and a brown to black discoloration shows through the skin. The presence of the rot is often exposed through breaking of the skin in the process of digging. Upon closer examination of affected tubers it will be found that the infection spreads more or less irregularly. In freshly dug tubers the rot may be described as a "soft rot" and is accompanied by a more or less putrid odor, but the blackleg as such is not to be confounded with this phase. Mixed infections, including the blackleg organism along with saprophytes and probably *Bacillus carotovorus,* are responsible for such conditions.

Dissection of tubers affected by the blackleg rot reveals the fact that the tissue may be involved to a considerable depth, extensive lesions often reaching the center. The color of necrosed tissues ranges from nearly normal to brown and black, but upon exposure to air these turn dark brown to black very rapidly (pl. 2, figs. 2 and 4). Advance of the rot is usually most rapid just beneath the epidermis (pl. 2, fig. 2), but in general spread in the tubers is not confined to any single region or tissue. Infected round tubers are often found to have a dark-colored hollow center.

A soft, cheesy rot caused by the blackleg bacillus is often seen among freshly dug tubers, though by some this type of rot is supposed to be due to "sunscald." The rot of tougher consistency found in affected tubers in transit or in storage is sometimes confounded with rots caused by *Fusarium* spp. Some very excellent illustrations of the tuber rot, including one colored plate, are those published by Shapovalov and Edson ('21).

Laboratory diagnosis.—To confirm the field observations it was necessary to make experiments in order to isolate the causal organism and prove its specific identity. Since sapro-

phytes quickly follow *Bacillus atrosepticus* in the tissues of both stem and tuber, it is often difficult to obtain the pathogen. By using extra precautions in carrying out the ordinary isolation methods one may obtain pure cultures of the causal organisms. A positive identification of the organism may then be made by careful comparison with the description of *B. atrosepticus* on p. 43.

ECONOMIC ASPECTS

Smith ('20) has come to regard the disease under consideration "as one of the most serious diseases of the potato." Earlier writers on the subject have unanimously emphasized its economic importance. Many cases are reported in the literature where upwards of 50 per cent of the plants in a field were destroyed by this disease. However, it appears that a loss of 5 per cent of the plants in a potato-growing district represents the usual amount of damage done by blackleg. In districts where the disease has been long known and where control measures have been intelligently applied, the losses have been greatly reduced, the average for the United States as a whole being about 0.5 per cent annually.

Dispersal and infection.—Probably the blackleg pathogen is most usually disseminated by the more or less general use of infected seed stocks. Morse ('16) mentions a striking illustration of this in a field in Idaho where he found blackleg which "undoubtedly came all the way from Scotland . . . in five years."

Without doubt the etiological agent is carried by wind and water, especially the latter, in the irrigated fields of the West. As pointed out by Smith ('20), invasion of the tubers through water-gorged lenticels can take place, but whether it does or not under natural conditions in the field is not known. The writer has planted healthy seed pieces in heavily infected soil but the disease did not develop in the plants thus grown. Similar results were recently attained by Shapovalov and Edson ('21). It appears to the writer that it still remains to be proven that the blackleg parasite can gain entrance to the vines or tubers through an unbroken epidermis.

The larvae of insects have been found by the writer working in and on the affected tissues, but there was no positive evidence that they were active agents in the dispersal of the disease. Von

Hegyi ('10) studied the disease on the continent of Europe, and since he found that every case of "blacklegged" stalks examined bore evidence of the attack of wireworms, he thinks insects are a positive factor in the invasion of the host. Paine ('17) believes that wireworms and biting insects are undoubtedly instrumental, under certain conditions, in introducing the parasite from the soil.

Geographical distribution.—The potato blackleg disease is now known to be widely distributed in the United States, Canada, and Europe. It was reported first in the northeastern United States and eastern Canada. Its spread, however, has been rapid, especially throughout the northern states. The disease has been reported from nearly every state in the Union. The records show, however, that it is essentially a cool-climate disease, therefore prevailing in more southerly districts only where potatoes are grown at high altitudes.

The writer finds that this disease in Montana and other sections of the Northwest is caused by the same organism known to prevail in the northeastern and North Central States. The recent work by Shapovalov and Edson ('21) proves that the same organism is widely distributed in the West.

Control.—Control measures should be inaugurated during the growing season. The writer strongly advocates the planting of a portion of the crop in a separate, if possible isolated, plot. This practice enables the grower to detect, remove, and destroy diseased hills at an early date.

Inspection and roguing of the plot (or field) should be begun soon after the plants are up and repeated at more or less frequent intervals throughout the growing season, especially during cool, rainy weather. Intelligent roguing will eliminate all hills where vines show any symptoms of the disease. Final inspections should be made just before and after digging. No tubers showing signs of rot or mechanical injury should be placed in storage. It is highly important that all seed stock be stored under favorable conditions. Slatted storage bins 8 or 9 feet high, 5 or 6 feet wide, and of any convenient length are ideal. In order to permit proper aeration of the potatoes, such bins should have false floors and must be separated from each other as well as from the walls of the cellar. The cellar should be fairly moist but provision should be made by proper regulation of ven-

tilators to prevent the atmosphere from becoming saturated. As nearly as practicable the temperature should be kept at about 38° F. in order to check sprouting. Butler ('19) has shown that potatoes kept at 40° F. will sprout after about 200 days, while at 35° F. sprouting is delayed indefinitely. Shapovalov and Edson ('19) showed that potato tubers which are in a wilted or softened condition when cut, due to the development of sprouts or improper storage, are very much more likely to be infected by fungous or bacterial parasites which exist in or may be introduced into the soil.

The seed pieces to be used for planting should be sorted and only sound, rot-free tubers used. Previous to cutting they should be treated in a 0.1 per cent corrosive sublimate ($HgCl_2$) solution or in a 1:240 formaldehyde dip. If the former is used, the tubers should be immersed for 1½ hours, then more of the dissolved mercuric chloride added at the rate of 1.5–2.0 grams (depending upon the amount of dirt accompanying the tubers) for each bushel treated. Avoid treating excessively dirty potatoes in the corrosive sublimate, and make up a fresh solution after having treated 50 bushels. If the formaldehyde solution is used the tubers should be immersed in the dip for about 1½ hours. Undoubtedly, a less lengthy immersion would be sufficient to kill all contaminating blackleg germs present. When the pathogen is lodged internally treatments sufficient to kill it would probably kill the buds also.

When and where practicable, planting should be done late enough to avoid having the crop sprout and struggle along in a cold, damp soil, since it has been shown that these conditions facilitate the development of the blackleg parasite. While it has not been conclusively proved that the blackleg parasite does not overwinter in the soil at times, it is nevertheless advisable to practice rotation of the crop, since some of the worst enemies of the potato accumulate in the soil and persist therein for long periods of time.

Tests made by the writer show that the blackleg bacillus is resistant to considerable extremes of cold. Test-tubes containing about 15 gms. of soil were thoroughly autoclaved and the sterility of the soil carefully tested before inoculation. Finally, a series of cultures thus prepared were inoculated with a freshly invigorated strain of the organism and placed out of doors for 24

hours. During this time the official minimum was —28° C. and the maximum —6.7° C. Soil cultures similarly inoculated were incubated at 28° C. The cultures exposed out of doors were placed on ice upon being brought in, in order to prevent excessively rapid thawing. Finally transfers were made to nutrient broth, and agar slants from all the cultures included in the experiment. The organism was recovered from all the cultures. The subcultures made from soil which had been frozen for 24 hours appeared to be as vigorous as any.

There are few satisfactory data on varietal resistance of potatoes to the blackleg pathogen. An early contribution to this phase of the problem was made by Appel ('03), who concluded that the thick-skinned, late varieties which were being grown in Germany were more resistant to the disease than the thin-skinned, starch-poor, early varieties. Morse ('17) reported that the Irish Cobbler and Green Mountain varieties were particularly susceptible to the disease. The Early Ohio, an early variety, is notably susceptible. The Russet Burbank and the Idaho Rural, varieties widely grown in the West, are commonly found affected with blackleg.

THE ETIOLOGICAL AGENT

The writer's studies on the etiology of the potato blackleg disease were begun in Montana some years ago. By the close of the summer of 1915 extended observation led to the assumption that the blackleg disease in Montana was quite similar to that occurring in some of the eastern states. In the meantime some 30 isolations were made at the Montana Experiment Station from potatoes affected with blackleg. The pathogenicity of the strains thus isolated was thoroughly tested for the second time in 1916, and a majority was found to be pathogenic and capable of causing typical symptoms of the disease. Comparative studies of the morphological, cultural, and physiological features of some of the above-mentioned strains were made by the writer. The results obtained led to the conclusion that all were essentially alike. An extension of these preliminary investigations was made, and cultures of the blackleg bacillus from Maine, Minnesota, and one from eastern Canada received under the name *B. solanisaprus* Harrison, were cultivated and compared with one another and with observations made previously on the Montana strains. While the observations made at this time were

not extended, the writer was led to assume that a single organism caused the potato blackleg disease in North America.

A study of the literature was begun, and it was soon learned that at least 4 different species of *Bacillus* had been described by as many authors as the cause of the disease under consideration.

By comparing the following descriptions it will be seen that the "species" in question do not differ markedly. In the summaries presented no attempt is made to follow the original form, but accuracy of statement is preserved.

Bacillus atrosepticus van Hall.—The following was prepared from a translation[1] of van Hall's ('02) original description. It bears comparison with that prepared for Morse ('17) by Dr. R. de Zeeuw.

Morphology: *B. atrosepticus* is a rod-shaped bacillus, occurring for the most part singly, rarely in pairs, in 2-day-old bouillon cultures at 27°C. Size variable; length 0.8–1.6 μ, breadth 0.2–.4 μ. Many zoogloea of 4–10 organisms. The bacteria are very active in a 24-hour culture of distilled water plus 0.025 per cent potassium phosphate and 0.25 per cent asparagine (27°C.). Material stained by Loeffler's method. Length of flagella 10–15 μ. Gram negative.

Cultural characteristics: Gelatin liquefied, rapidity variable. Growth on malt gelatin and malt agar very weak. Milk coagulated. Growth best at top of meat agar stab.

Physiology: Growth very strong at 27°C. Thermal death point 51–52°C. Facultative anaerobe. Reduction of methylene blue weak. Nitrates reduced to nitrites. Sodium selenite reduced rapidly. No diastatic action. No indol production. No H_2S produced in broth cultures. The organism is a weak gas producer (except when mannite is present). Gas produced from glucose, saccharose, and mannite in a medium made by adding to "due water" (a filtered water) 0.025 per cent K_2HPO_4, 1 per cent peptone and 3 per cent of sugar. No gas from lactose and glycerin in same medium. Growth slow at first in bouillon acidified to a reaction of 0.5 per cent normal with citric and malic acid. Organism grows poorly when transferred from dried cultures: thought not to be resistant to drying. Pathogenic to potato stems and tubers. Index No. 5312-32120-2121.[2]

Bacillus phytophthorus Appel.—The following is summarized from Appel's ('03) diagnosis:

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[1]My thanks are due to Dr. J. C. Th. Uphof for assistance rendered in translating Part III of van Hall's ('02) dissertation.

[2]"Index Number" digits throughout this paper are arranged according to the Descriptive Chart indorsed by the Society of American Bacteriologists, Dec. 30, 1920.

Morphology: *Bacillus phytophthorus* a fairly thick, colorless, rod-shaped organism. Breadth averages 0.8 μ, length 1.2–1.5 μ. Usually occurring singly, occasionally in pairs. Motile by means of (usually six) peritrichic flagella, stained by Peppler method. Gram negative.

Cultural features: Liquefaction of gelatin saccate. Gelatin colonies small, yellow-white, with entire margin. Agar colonies small, glistening, bluish-opalescent. Growth on agar stroke rapid, moderate to abundant. Upon sterilized potato plugs a weak honey-yellow growth develops. On sterile raw potato slices, growth and browning in 15–18 hours after inoculation. In 2 days middle line sinks and surrounding tissue becomes dark on upper surface. A liquid is pressed out. Growth in potato juice rapid and abundant. Growth in nutrient broth moderate, turbid and sediment.

Physiology: Gas from sucrose and maltose. No gas in shake cultures of sugar agar. Nitrates reduced to nitrites. Milk coagulated slowly, acid (?) curd. Pathogenic to potato stems and tubers. Index No. 5?12-32120-???1.

Smith's description of B. phytophthorus Appel.—Inasmuch as Appel's ('03) original diagnosis of his species is meager, the following compilation of E. F. Smith's ('20) description is presented:

A white, rapid-growing, non-sporiferous, Gram-negative, motile, peritrichiate-flagellate, promptly liquefying, nitrate-reducing, aerobic and facultative anaerobic, acid-forming, gas-forming, milk-curdling (by formation of an acid), dry-air-sensitive, rod-shaped or filamentous schizomycete, forming quickly on agar plates circular, grayish white, well-developed colonies; on very thin-sown gelatin plates characteristic, rapid-growing, big, circular, opaque white colonies. Organism white on most media but on Soyka's milk rice it is pale pinkish cinnamon. Bouillon clouded very quickly and gelatin stabs develop a prompt funnel of liquefaction. The organism does not form indol, and does not grow in Cohn's solution. It produces a non-volatile acid from dextrose, saccharose, lactose, galactose, and maltose, and small quantities of gas from inosit (muscle sugar), lactose, and mannite. Pathogenic to potato shoots. Index No. 5312-32120-?212.

Bacillus solanisaprus Harrison.—Summary given herewith is compiled from Harrison's ('07) original description:

Morphology: Bacillus of variable size. From 24-hour-old beef agar spores not seen; flagella peritrichic; organism Gram negative.

Cultural features: Growth in agar abundant, glistening, opalescent; persistent growth on potatoes; uniform growth in gelatin cultures, filiform liquefaction; strong growth in nutrient broth; ring in surface growth; clouding strong, fluid turbid, fine sediment; growth in gelatin colonies slow, round to elliptical; agar colonies round to lenticular, shiny, entire.

Physiology: Prompt coagulation of milk, extrusion of whey in 3 days, a few gas bubbles visible; litmus milk acid. Strong growth in Uschinsky's solution. In fermentation tubes gas was produced in bouillon containing mannite and lactose; growth in closed arm, with production of acid in each of the following: dextrose, saccharose, lactose, maltose, glycerin, mannite, and levulose. Nitrates in nitrate broth were reduced, nitrites present, indol production moderate to feeble; vitality on culture media long; thermal death point 54° C., optimum temperature for growth 25–28° C., growth slight at 37° C.; maximum temperature for growth 37.5°C., minimum temperature for growth about 0° C. Pathogenicity proven to following vegetables: potato, tomato, Jerusalem artichoke, and others; also to living plants of potato, tomato, common red pepper, and slightly in cucumber and physalis. Index No. 5312–32120–1212.

Bacillus melanogenes Pethybridge and Murphy.—The account presented below is summarized from Pethybridge and Murphy's ('11) original diagnosis:

Morphology: Vegetative cells 0.7–0.9 μ × 1.3–1.8 μ, found most frequently in pairs. Flagella peritrichic (fewer than in *B. solanisaprus* Harrison). No endospores. Gram's stain negative.

Cultural features: Nutrient broth clouded, more or less sediment, fluid turbid, no pellicle. Organism did not form a distinct ring on surface of potato juice. Gelatin colonies round. Growth best at top in gelatin tubes, some growth along needle track Gelatin liquefied. On potato plugs growth abundant, and chromogenesis yellowish.

Physiology: Facultative anaerobe. Milk acid curd, curd not very compact. No indol produced, nitrates reduced to nitrites with formation of gas. Diastatic action on starch. Acid and gas in fermentation tubes, in broth plus glucose, lactose, and saccharose. No acid from glycerin. Pathogenic to potato.

They comment upon the marked resemblance of their organism to *B. phytophthorus* Appel and state that they were tempted to regard it as a variety of the latter. Index No. 5312–3211?–1111.

After comparing the contributions of these earlier investigators, and in the light of the observations which I had previously made, I planned a thoroughgoing study of the relationship of the blackleg pathogen. To this end it was thought best to make careful comparative studies of a number of strains, including if possible the 4 species described above.

Source and history of the cultures used.—Some difficulty was experienced in obtaining authentic and viable subcultures of the organisms described by van Hall, Appel, Harrison, and Pethybridge and Murphy. Cultures of these organisms, however, were

finally obtained.[1] These were supplemented by cultures of the blackleg parasite isolated in Montana, Minnesota, and Maine. For the sake of convenience all of the subcultures collected were designated by a number. In order to facilitate matters, all records of studies made on the strains selected were kept under an assigned number.

Below is given a brief note on the source and history of the several cultures used more or less extensively throughout the writer's comparative studies. The first 5 enumerated were from isolations made in the Botany Department at the Montana Experiment Station and were selected from isolations made from potatoes affected with the blackleg disease obtained in some of the more important potato-growing sections of that state.

No. 160.1. Isolated 8/14/15, from tuber affected with black rot. Material from near Bozeman, Mont. Pathogenicity established. Still virulent late in 1917.

No. 170.3. Isolated 8/22/15, from tuber affected with black soft rot. Material from near Lewistown, Mont. Pathogenicity established. Still virulent late in 1917.

No. 180.2. Isolated 7/24/16, from black, cortical lesions on stem. Material from near Kalispell, Mont. Pathogenicity established. Still virulent late in 1917.

No. 183.2. Isolated 8/27/16, from black cortical lesions on stem. Tubers on same vine affected with blackleg rot. Pathogenicity established. Still virulent late in 1917.

No. 187B.1. Isolated 9/2/16, from tuber with superficial black rot lesions. Pathogenicity established. Still virulent late in 1917.

No. 191. Kindness of W. J. Morse. His "*B. phytophthorus* from Appel." Pathogenicity not established by the writer. Morse found it non-pathogenic.

No. 193. Kindness of W. J. Morse. His "III A," isolated by him in Maine, Aug., 1908. Found by the writer to be pathogenic. Still virulent late in 1917. Used by Morse in his studies ('17).

No. 194. Kindness of W. J. Morse. His "SE." Isolated by him in Maine, Aug., 1908, from a "potato stem showing a very rapid soft rot." Found by the writer to be pathogenic. Still virulent in late 1917. Used by Morse ('17).

[1] My thanks are especially due Dr. W. J. Morse and Dr. B. M. Duggar for very material assistance rendered in supplying certain of the cultures used in this work.

No. 195. Kindness of W. J. Morse. His "II P." Isolated by him in Maine, Aug., 1908, "from typical blackleg plants". Found by the writer to be pathogenic to potato tubers. Still virulent late in 1917. Used by Morse ('17).

No. 196. *B. solanisaprus* Harrison. Kindness of W. J. Morse, who used this strain in his studies ('17). The culture was originally procured from S. F. Edwards, Ontario Agr. Coll., in March, 1909. Found by the writer to be pathogenic to potatoes. Virulence in 1917 good.

No. 197. *B. atrosepticus* van Hall. Kindness of W. J. Morse. Previously studied by him ('17). This strain Morse "received under that name from Kral's laboratory[1] in 1910. . . . . At first it showed weak pathogenicity to potato tubers, and repeated inoculations to growing stems failed to produce the disease *until the present summer* [1916]." Found by the writer to be pathogenic, though, if anything, less strongly so than some of the others. Still virulent late in 1917.

No. 198. *B. melanogenes* Pethy. and Murphy. Kindness of W. J. Morse, who "received this from Dr. Pethybridge himself in 1911. . . . . It also has produced active decay of tubers and blackleg of the stem upon inoculation." Used by Morse ('17). I found it to be pathogenic. Still virulent in Nov., 1917.

No. 200. *"B. phytophthorus"* from Minnesota. Kindness of E. C. Stakman. Isolated 1915. The writer found it to be pathogenic to potato tubers. Still virulent in 1917.

No. 201. *B. phytophthorus* Appel. Kindness of B. M. Duggar who obtained the culture from E. F. Smith. The latter procured the strain from Dr. Aderhold in Berlin about 1906. It is presumed that Smith's culture was a transfer from Appel's original culture. The writer found it to be pathogenic. Still virulent in 1917.

No. 202. *"B. solanisaprus."* Kindness of D. H. Jones who wrote that "the transfer purports to be from original strain of *B. solanisaprus* . . . . received in Oct., 1916, from the American Museum, to which Dr. Harrison had formerly sent a culture." Found by the writer to be pathogenic. Still virulent in late 1917.

II. Comparative Studies of Causal Organisms

Invigoration of cultures.—The cultures used by the writer were growing on beef agar at the time the comparative studies presented below were begun. Some had been previously cultivated on agar and some in nutrient broth for greater or less length of time. To begin with, all were plated out to insure purity of the culture and then each one was invigorated by trans-

[1] Kral's Bakteriologisches Laboratorium, Prague, Austria.

ferring to nutrient broth and cultivating at 25–27° C., on 3 consecutive days. All the cultures so treated responded promptly.

Pathogenicity.—Testing the pathogenicity of the strains to be used proved to be a considerable task. A number of trials were made before satisfactory proof was obtained of the pathogenic character of all of the strains in use. In the first series of tests potato tubers were used. On the whole, sound, smooth tubers of some thin-skinned variety, such as Early Ohio, provided the most satisfactory material for artificial inoculation experiments. Thick-skinned, late varieties, as the Russet Burbank, can be used. Tubers with a relatively high sugar content proved to be the best for this work (cf. Carbohydrate Utilization, page 45). On this account it is well if possible to obtain growing tubers, or else those from storage which are held till sprouts begin to develop.

Method.—Selected tubers were thoroughly washed by scrubbing in warm water, then soaked 3–4 hours in clean warm tap water, finally disinfected by immersing for 15 minutes in a 0.1 per cent aqueous solution of mercuric chloride. Immersion in this disinfectant for a longer period of time is not necessary. Moreover, it was found when the tubers were soaked for 1½–2 hours that the chemical remained in the outer layers of the skin, to the extent of making it difficult, if not impossible, to remove all of it by washing. Not infrequently the effects of a long soaking were manifest by inconspicuous lesions in the surface layers. Clean ground-glass plates, watch-glasses, and bell jars were made ready and sterilized in advance by washing in the bichloride solution. The tubers were then removed from the disinfecting solution, thoroughly washed in sterile tap water, and placed on watch glasses under the bell jars.

Preliminary experiments demonstrated the desirability of maintaining a nearly saturated atmosphere surrounding the tubers during incubation for 4 or 5 days. This requirement was easily satisfied by lining the inside wall of each bell jar with sterilized filter-paper wet with sterile water. If the atmosphere of the room is dry and cool it will be found best to place the inoculation chambers in an incubator kept at 22–25° C. All but one of the tubers under each bell jar were inoculated. Inoculations were made in a clean damp culture room. It was accomplished by placing a drop of the suspension from a recently in-

vigorated culture in a depression, usually near an eye, and stabbing through the inoculum 3 or 4 times with a sterile needle. The control tubers were pricked in similar places with a sterile needle. Inoculation courts were marked with an indelible pencil by tracing a good-sized circle around the spot where the tuber was pricked. Check courts were marked by a square.

Observations and conclusions.—The inoculation sets thus prepared were closely observed every day for 10 days, then with less frequency for over a month.

The number of days elapsing before signs of infection were visible varied, in the several experiments performed, from 2 to 6 days. Infection was in general more prompt where tubers having a high sugar content were inoculated, where the set incubated at around 23° C., and where the moisture content of the atmosphere surrounding the tubers was kept high. Observations made by the writer on the pathogenicity of several strains of the potato blackleg parasite suggest the conclusion that the "incubation period" in plants is markedly influenced by environmental conditions. The "incubation period" did not appear to be marked by a definite "turning point," as is the case in many animal diseases.

The writer concluded that all the strains being tested were pathogenic (cf. tables I and II). The nature and development

TABLE I

TABULAR SUMMARY

SOURCE AND HISTORY OF CULTURES USED

Cult. No.	Isolated	Source	Pathogenicity		Comparative virulence
			Stems	Tubers	
160.1	Tuber 8-14-'15	Montana	Marked	Strong	Average
170.3	Tuber 8-22-'15	Montana	Marked	Strong	Average
180.2	Stem 7-24-'16	Montana	Strong	Marked	Average
183.2	Stem 8-27-'16	Montana	Marked	Marked	Average
187B.1	Tuber 9-2-'16	Montana	Strong	Strong	Strong
193.	"Plant" Aug., '08	Maine, Morse's IIIA	Strong	Strong	Average
194.	"Stem" Aug., '08	Maine, Morse's SE	Yes	Yes	Average
195.	"Plant" Aug., '08.	Maine, Morse's IIP	Strong	Yes	Average
196.	Before Mar., '09	*B. sol.* Ontario, Can.	Marked	Marked	Average
197.	Before 1910	*B. atro.* Kral's lab.	Marked	Yes	Average
198.	By P. & M. 1911	*B. mel.* fr. Pethy. Ireland	Yes	Yes	Low
200.	"Blackleg" 1915	Minnesota	No data	Yes	Low
201.	Before 1906	*B. phyto.* fr. Germany	Marked	Marked	Strong
202.	About 1907	*B. sol.* Am. Museum	No data	Strong	Average

of rot lesions in the tubers varied considerably under the different conditions of the artificial inoculation experiments. Lateral spread from the points of inoculation seldom took place to the extent it does in tubers invaded at the stem end and under natural conditions. The depth to which the lesions extended was usually to a point considerably beyond the depth of the needle prick, occasionally to the opposite side of the tuber. The character of the rot developed in the tubers inoculated artificially varied somewhat. Sometimes the affected tissues were quite moist and soft. Again, the tissues attacked were quite dry, being more or less spongy or cheesy in texture. Diseased material exhibited a more or less putrescent odor but in no case did inoculation with pure cultures effect a profound, gray, slimy, obnoxious, gaseous, soft rot. Variations, such as those mentioned, appeared to be associated to a considerable degree with (1) the available sugar content of the tuber, (2) the moisture content of the tuber, and (3) with certain environmental factors, notably temperature and humidity. The lesions in immature potatoes were generally more profound and the affected tissues softer than those in ripe tubers, particularly of a late, hard, starch-abundant variety A drop of inky black liquid was exuded at the points of inoculation in many cases. Occasionally a slight bulging of the tissues occurred at these points. When affected tubers were cut or broken open the diseased tissues were characteristically dark-colored, becoming brown or black upon exposure to the air.

Additional observations.—To extend the above observations on pathogenicity of the various strains at hand, the artificially inoculated tubers were cut into seed pieces and planted in pots in the greenhouse. In many instances the seed piece was entirely consumed by the blackleg rot before sprouts started. In a few cases, however, sprouts developed, only to be rapidly invaded by the pathogen and killed. The case of culture 160.1 is typical. Four stalks emerged from the ground but by the time they had grown to be from 3 to 6 inches tall (30 days after planting the seed pieces), the parasite invaded the stem tissues. The infection extended rapidly upward. At first the diseased tissues (cortical) appeared water-soaked. The tissues became dark-colored over night, and in a day or two the plants were prostrated (pl. 1, fig. 3). What proved to be a pure culture (plates) was recovered from this material. This was later used for inoculation work and found to be pathogenic in tubers.

Some experiments on the pathogenicity of the potato blackleg parasite performed at later dates furnish further proof of the pathogenicity of the strains under observation, and will be reported here. A brief survey of the results obtained in one of these experiments (II of 7/21/17) appears in tabular form below (table II). Tubers of the Russet Burbank variety were used. They had been kept in storage nearly a year. The tubers were washed and disinfected in the manner described previously. A number of selected tubers were then cut into seed pieces, each weighing 2 to 3 ounces. Three seed pieces were then inoculated with each of the several strains of the organism at hand. Two were planted in pots, the third being placed in a moist chamber kept at room temperature (20–24° C.)

TABLE II

PATHOGENICITY OF STRAINS OF B. ATROSEPTICUS EMPLOYED

Strain No.	Pathogenicity to seed pieces planted in soil	Pathogenicity to seed pieces kept in moist chamber
160.1	Positive (sprouts involved)	Negative (no infection, piece dry)
170.3	Positive (no sprouts)	Negative (no infection, piece dry)
180.2	Positive (no sprouts)	Positive (black rot)
183.2	Positive (no sprouts)	Positive (black rot)
187B.1	Positive (no sprouts)	Negative (no infection)
191.	Negative (sprouts)	Negative (non-pathogenic)
193.	Positive (sprouts)	Positive (black rot)
194.	Negative (sprouts)	Positive (black rot)
195.	Negative (sprouts)	Negative (no infection)
196.	Positive (no sprouts)	Positive (black rot)
197.	Negative (one sprout)	Positive (black rot)
201.	Positive (no sprouts)	Negative (no infection)

Evidently all the strains used were still virulent.

Among other things this experiment shows that the blackleg organism may destroy infected seed pieces before sprouts develop. In other cases the infectious material may be rendered inocuous or even killed by drying and by other agents. These conclusions are emphasized by experiments performed and reported above.

An experiment begun in August, 1917, showed that the strains previously listed were pathogenic to potato tops; that is, all introduced artificially gave rise to typical blackleg lesions in the stems and in leaf petioles.

A series of experiments was planned during the summer of 1917 in order to throw light on the question of whether potatoes planted in a soil contaminated with the blackleg parasite would

become affected with the disease. Only one trial was made, and while the results obtained do not in themselves warrant drawing a conclusion, they are of interest when compared with those reported by Shapovalov and Edson ('21). Ordinary seed pieces from sound, hard, healthy potatoes were planted in soil which had been previously heavily seeded with the blackleg germ by planting with tubers affected with the disease. The seed pieces sprouted and the plants therefrom grew to be of good size. No signs of infection by the blackleg germ were to be found in the seed piece, on the roots, or in the tops.

METHODS AND MEDIA

Certain of the bacteriological methods recommended by the American Public Health Association ('12) were followed as closely as possible by the writer in the prosecution of his studies of the cultural characteristics and physiology of the blackleg strains selected for comparison. In some cases this was not practicable or even possible on account of the fact that certain reagents were not available, due to conditions brought on by the World War. For this reason, a rather complete statement is given below of the media and methods employed, thus making it possible to duplicate both.

The present-day methods for determining H-ion concentration of bacteriological media were not in general use at the time the writer carried out his comparative studies. The ordinary media used were titrated with N/20 NaOH, using phenolphthalein as an indicator. In most cases the reaction of the medium was not adjusted. Wherever stated the reaction is given as "+ 7" etc., where the addition of 7 cc. of normal alkali per 1000 cc. would render the medium neutral to phenolphthalein. A "O" indicated that the medium as used was neutral to phenolphthalein. The precaution of having all flasks, beakers, test-tubes, etc. thoroughly clean was taken at all times.

Media.—Steps in the preparation of the media are briefly indicated below:

NUTRIENT BROTH

(1) To 1000 cc. distilled water add 50 gms. "Bacto" Beef Extract; (2) heat the mixture gradually up to 70°C. during about 1 hour; (3) boil for a few minutes to coagulate precipitable proteins; (4) make up the water lost by evaporation; (5) filter through layers of clean cheese-cloth; (6) cool to about 60°C. and add 1 per cent "Difco" peptone, ½ per cent NaCl, and dissolve; (7) filter through

paper; (8) titrate, using phenolphthalein as an indicator; (9) determine reaction and adjust if necessary; (10) sterilize in autoclave at 15 pounds for 15 minutes.

BEEF AGAR

(1) Nutrient broth (steps 1–6) is brought to a boil; (2) while boiling add 1 per cent "Bacto" agar, sifting it over the surface; (3) when agar is completely dissolved, cool and clarify in usual manner; (4) determine reaction and adjust if necessary; (5) filter; (6) sterilize in autoclave.

GELATIN

(1) To nutrient broth already prepared (steps 1–6), add 10 per cent "Bacto" gelatin and dissolve at a low temperature (60–70°C.); (2) determine reaction and adjust if necessary; (3) filter, tube, and sterilize at 15 pounds for 15 minutes.

DUNHAM'S SOLUTION

Make a paste of 10 gms. peptone and 5 gms. salt in a small quantity of water; then add sufficient water to make total used 1000 cc. Heat in flowing steam for ½ hour, then boil for 10 minutes over free flame. Make up water lost by evaporation. Filter through paper and tube. Sterilize in autoclave at 15 pounds for 15 minutes.

CARBOHYDRATE BROTH

(1) To nutrient broth already prepared (steps 1–6) add 1 per cent by weight of the chemically pure carbohydrate; (2) determine and record reaction; (3) filter, tube, and sterilize in autoclave at 15 pounds for 15 minutes.

MILK

The milk used was freshly drawn into a sterile bottle. After standing about an hour it was centrifuged and the cream skimmed off. Samples were titrated, using phenolphthalein as an indicator, and the reaction was determined to be "+20". The reaction was not adjusted. The medium was tubed promptly and sterilized by exposing tubes in flowing steam for 20 minutes on 3 consecutive days.

LITMUS MILK

To part of the fresh milk (see above), was added 2 per cent by volume of a saturated aqueous litmus solution (Merck's reagent, 1 gm. to 15 cc. H_2O). This medium was tubed immediately and sterilized by exposure to flowing steam for 20 minutes on 3 consecutive days. Both milk media were kept 4 or 5 days before being inoculated.

RAW POTATO PLUGS

A considerable number of raw potato plugs were prepared, observing all possible antiseptic precautions. The lot was kept several days in order to insure the removal of any contaminations that might appear.

RAW POTATO DISKS

Sterile disk-shaped slabs from raw potatoes were successfully prepared in the same manner as the preceding lot. They were placed in sterile Petri dishes on a piece of sterile wet filter-paper. On the whole, these disks proved to be more usable than the raw potato plugs.

COHN'S SOLUTION

Dissolve 5.0 gms. KH_2PO_4, 5.0 gms. $MgSO_4$, 10.0 gms. $(NH_4)_2$-$C_4H_4O_6$, and .5 gm. $(Ca_3(PO_4)_2)$ in 1000 cc. distilled water, and filter through paper. Sterilize in autoclave at 15 pounds for 15 minutes.

USCHINSKY'S SOLUTION (MODIFIED)

The modified solution was made up according to the formula given by Smith ('05).

EHRLICH'S INDOL TEST SOLUTION

Solution I.—Para-dimethyl-amido-benzaldehyde, 4.0 gms.; 95 per cent alcohol, 380 cc.; HCl (conc.), 80.0 cc.

Solution II.—Saturated aqueous solution of potassium persulphate.

To about 10 cc. of the liquid culture (preferably in Dunham's solution and 10 days old), add 5 cc. of Solution I, then 5 cc. of Solution II, using separate clean pipettes. Shake the mixture. The reaction may be accelerated by heating to about 70°C. The appearance of a red coloration which increases in intensity indicates the presence of indol.

NITRATE BROTH

Filter, tube, and sterilize in the autoclave 1 gm. peptone ("Bacto"), 0.2 gm. potassium nitrate cp. (Merck), and 1000 cc. distilled water.

REAGENTS AND TEST FOR NITRITES

Test for nitrites in nitrate broth culture on fifth day by adding: (1) one cc. of a 1 per cent potato starch water; (2) one cc. of a freshly prepared KI water (i.e., KI, 0.2 gm. in 50 cc. distilled water); (3) a few drops of strong H_2SO_4, (i.e., conc. H_2SO_4, 1 part, distilled H_2O, 2 parts). The appearance of a blue-black coloration in 10 to 15 minutes was taken as positive evidence of the presence of nitrites.

DETAIL OF COMPARATIVE STUDIES

MORPHOLOGY

A detailed study of the morphological characteristics of the several strains at hand was not attempted by the writer. However, it was determined that each was a small, short, actively motile, rod-shaped organism, easily stained by the ordinary aniline dyes. Pairs of the organism occurred in preparations made from fresh cultures on agar slants. No spores were found in any of the cultures, and all were determined to be Gram negative.

Cultural features and physiology.—In prosecuting the general plan outlined for making comparative studies of the cultural features and physiology, it was deemed wise to give particular attention and painstaking effort to the study of those items which are most commonly made use of in establishing bacterial species.

In recent times the response of the microorganism, as well as the reactions set up by it, in the presence of carbohydrates, has been stressed in attempts to distinguish between closely related species. On this account, and because of the fact that earlier students of the potato blackleg parasite report a great variety of conclusions as to the ability of this organism to ferment certain carbohydrates, the writer made exhaustive studies upon the gas- and acid-producing function of the blackleg bacillus. Details of the comparative studies appear on the several tables presented herewith and in the paragraphs below.

Nutrient broth:— Clouding prompt, persistent; medium becoming slightly turbid; sediment compact, granular, somewhat viscid; surface growth a ring, though under certain circumstances a light pellicle may form; no chromogenesis; odor absent; color of medium unchanged.

Earlier students of the blackleg bacillus did not wholly agree as to cultural features exhibited by this organism when grown in nutrient broth. The several strains studied were grown in 3 nutrient broths: (A) "Bacto" beef and "Bacto" peptone, (B) Liebig's beef extract and Witte's peptone, (C) fresh meat infusion and Witte's peptone, and all incubated at 27–28° C. All (except No. 191) were very similar in their response in nutrient broth. In those grown in "Bacto" beef and "Bacto" peptone (except Nos. 191 and 198) the surface growth was a ring, clouding was moderate, sediment granular-viscid, the medium slightly turbid, and no color. In the case of 191 the surface growth was a membrane, and in 198 a light pellicle with moderately strong clouding. When the strains were grown in Liebig's beef extract and Witte's peptone the surface growth was a ring (except No. 191 which was a membrane), clouding moderate, sediment granular viscid, medium slightly turbid, chromogenesis none. In those grown in fresh meat infusion and "Bacto" peptone, except Nos. 160.1 170.3, 187B.1, 191, 197, and 201, the surface growth was a pellicle, the clouding strong, the sediment granular-viscid, medium slightly turbid, and chromogenesis none. Nos. 160.1 and 187B.1 showed the surface growth a wide ring, and

moderate clouding; No 170.3 and 197, moderately strong clouding; No. 191, the surface growth a membrane with moderate clouding; and in No. 201 the surface growth was a light pellicle.

Observations recorded upon the cultural features exhibited by the different strains (except No. 191) as grown in these broths suggest a clue as to the disagreement between all who have cultivated the blackleg bacillus as to the character of the surface growth. It was found that the clouding was stronger and the surface growth heavier (a pellicle) in the nutrient broth made from a fresh meat infusion and "Bacto" peptone than in either of the other two nutrient broths used. Moreover, the growth response in the broth made with "Bacto" beef and "Bacto" peptone was somewhat more luxuriant than that in the beef extract and Witte peptone broth. The writer concludes that strain No. 191 is the only one of the 12 studied in broth that exhibits dissimilarity.

Agar stroke.—Growth moderate, filiform, somewhat raised, with smooth surface and glistening luster, translucent and somewhat iridescent, non-chromogenic; consistency butyrous, some quite viscid; odor absent; medium (color) unchanged.

On agar slants the cultural features were identical for the strains studied, except No. 191, where the form was spreading and effuse, the surface smooth, and luster dull.

Potato (cooked).—Growth moderate to abundant, filiform at first, spreading after a few days, slightly raised and flat, glistening, with a smooth to slightly contoured surface; chromogenesis yellowish white; odor not strong nor characteristic; color of medium slightly browned.

Special attention was devoted to the cultivation and study of the cultural features of the blackleg strains on sterilized potato plugs. This was done because of the importance attached by some authors to the cultural characteristics on this medium, in the differentiation of their "species" from others. All the strains studied comparatively by me were quite alike (except No. 191), in so far as can be detected from a study of the growth and cultural features on sterilized potato plugs. In No. 191 the growth was scanty, form spreading, surface smooth, dull, and colorless.

Potato (raw).—Raw potato slabs aseptically prepared as outlined in a preceding section were used. Preliminary tests indicated that infection and growth by the organism took place when the atmosphere of the culture chamber (Petri dish) was moist and when incubated at about 26° C.

The following strains were tested: Nos. 160.1, 170.3, 180.2, 183.2, 187B.1, 191, 195, 196, 197, 198. All responded and grew very similarly, except No. 191. In the case of the other 9 strains a considerable browning of the tissue approximate to the lines of inoculation was plainly visible at the end of the first day. A small quantity of dark brown to black liquid collected adjacent to the line of growth. By the end of a week abundant development of the cultures had taken place, forming a grayish white slime surrounded by a dark-colored border. The tissues at the margin of the growth were dark brown to black in color. Underneath the bacterial growth they were somewhat collapsed, so that the culture was growing in a shallow depression. No growth took place on the uninoculated slabs kept as controls.

Agar colonies (tubes inoculated at 28° C.).—Colonies rather small (3–15 mm. in 15 days); growth moderately rapid; form round to somewhat irregular; surface smooth; elevation raised and flat; edge entire, becoming undulate or lobed; internal structure, when magnified about 50 times, finely to coarsely granular; chromogenesis none; submerged colonies small, biconvex to ovoid, or nearly round; color of medium unchanged.

In strain No. 191, the colonies were large; surface smooth; elevation effuse; edge erose; and internal structure fine. This was the only one of the 11 planted in agar plates that developed markedly different features. In fact, one is led to the belief that no more than 1 species could possibly be represented by the 10 strains in question.

Gelatin colonies (incubated at 18–23°C.).—Form round to somewhat irregular; edge entire, often becoming undulate; liquefaction first noticeable, as a rule, about 24 hours after planting, proceeds rapidly, and is saucer-shaped; chromogenesis none.

Here again the cultural features and the physiological response as manifested by liquefaction were identical for all strains, except No. 191. In this case there was no liquefaction, and the colonies were larger. At about 21° C. colonies developed quite promptly in gelatin, and averaged 5–20 mm. in size, which was considerably larger than the agar colonies.

Gelatin stab.—Growth best at top; line of puncture filiform, often slightly beaded; liquefaction begins in about 24 hours and at 20°C., at first crateriform or napiform, becoming infundibuliform or stratiform, complete as a rule at end of third week, when culture is aerated by shaking occasionally; the liquid gelatin becomes cloudy and often a ring; sometimes a pellicle develops on the surface.

With a single exception (No. 191) the strains cultivated by stabbing gelatin plugs grew and responded almost identically as may be seen by glancing at table III.

Milk.—Fresh milk (+20) incubated at 28° C. is promptly coagulated. The amount of acid developed by the organism increases steadily for 18 days at least. Peptonization slow, begins in 2–3 days. In similar cultures to which litmus was added, a marked bleaching of the indicator took place by the end of the eighth day, as a rule. The addition of 2 per cent (by volume) of a saturated solution of Merck's purified litmus had no noticeable inhibitory effect upon the growth of the strains of the blackleg bacillus tested.

It may be noted that considerable difference appears in the descriptions published by earlier authors as to the cultural features and physiology of the blackleg bacillus when grown in milk. The writer failed to discover any real differences among the strains cultivated by him except in the case of strain No. 191 which, as has appeared heretofore, is non-pathogenic and otherwise quite different in its cultural features and physiology (see table III).

Uschinsky's solution.—Growth copious; fluid not viscid.

In this solution in which the nitrogen is supplied by organic compounds (see p. 28) all strains cultivated developed similarly except No. 191, which grew more copiously than the others with the development of a pellicle upon the surface of the medium.

TABLE III

CULTURAL FEATURES AND PHYSIOLOGY

GELATIN STAB*		REACTION 0		INCUBATED AT 20° C	
Culture	Growth	Line of puncture	Liquefaction		
			Type	Begins	Complete
				days	days
160.1	Best at top	Fil.-beaded	Strati.-infund.	2	22
170.3	Best at top	Fil.-beaded	Stratiform	1	22
180.2	Best at top	Fil.-beaded	Napi.-strati.	1	22
183.2	Best at top	Fil.-beaded	Napi.-strati.	1	22
187B.1	Best at top	Fil.-beaded	Crater.-strati.	2	22
191.	Best at top	Filiform	None		
194.	Best at top	Filiform	Strati.-infund.	1	22
195.	Best at top	Filiform	Strati.-infund.	1	22
196.	Best at top	Fil.-beaded	Napi.-strati.	1	22
197.	Best at top	Fil.-beaded	Crater.-strati.	2	22
198.	Best at top	Fil.-beaded	Napi.-strati	1	22
201.	Best at top	Fil.-beaded	Crater.-strati.	2	22

TABLE III—(Continued)

MILK	REACTION 20			INCUBATED AT 28° C.	
Culture*	Plain milk				Litmus milk
	Coagulation begins	Reaction		Peptonization begins	Reduction of litmus‡
		6 days	18 days		
	days			days	days
160.1	2	+23†	+47†	3	7-8
170.3	2	+33	+52	3	7-8
180.2	2	+36	+46	3	7-8
183.2	2	+34	+50	3	7-8
187B.1	2	+39	+49	3	7-8
191.	None	− 8	+24	None	None
194.	2			3	7-8
195.	2	+32	+53	3	7-8
196.	2	+34	+52	3	7-8
197.	2	+29	+39	3	8-9
198.	2	+10	+35	3	12
201.	2	+35	+51	3	7-8

* None of the strains changed the color of the medium.

† Figures represent increase or loss in degrees on Fuller's scale over controls titrated on same day.

‡ The indicator had very little, if any, effect on the growth.

Cohn's solution.—No growth.

Indol production.—Negative.

A survey of the published descriptions of the potato blackleg bacillus reveals the fact that half of the authors conclude that this organism produces indol, while the others arrived at an opposite conclusion. Special pains were therefore taken by the writer to determine this point accurately.

Kligler ('14), Lewis ('15), and others have criticized the use of the Salkowski-Kitasato method (conc. H_2SO_4 and $NaNO_2$) on account of its unreliability. Kligler ('14) states that this test should be discarded, since a red coloration is frequently obtained which is not due to indol; also because the reaction in cultures which really produce indol is not constant. Ehrlich's method of testing for indol production was used by the writer in his experiments. This test will detect indol in a dilution of 1:1,000,000. It has been shown that it is 10 times more delicate than the older test mentioned above. In order to make his tests even more searching, the writer employed both "Bacto" peptone and Witte peptone in making the Dunham's solutions used as culture media. All 12 strains of the blackleg bacillus were carefully tested, using controls inoculated with *Bacillus coli,* as well as blanks. A pink coloration did not appear in a single culture

except those in which *B. coli* was planted. Incidentally, it may be stated that the strain of *B. coli* used gave rise to a stronger color reaction in the Witte peptone solution than in the "Bacto" peptone solution.

Heating at 70° C. for a few minutes did not bring out the slightest trace of pink coloration in any of the cultures inoculated with strains of the blackleg organism. Cultures kept for 2 months were also tested, and negative results were obtained as before. In these cultures a yellowish or faint brown coloration was noticed after adding the test solution. Upon looking across the surface of the liquid against a dark background, the suggestion of a very pale wine coloration was obtained which in the absence of the positive test color might be mistaken for indol production. Possibly some of the authors who have reported that the blackleg bacillus produced indol were led astray by the appearance of similar color reactions in their test cultures. Repeated warnings have been given against accepting such colorations as a positive indication of indol. When compared with the pink color appearing in cultures of *B. coli* tested for indol, one is not likely to make an erroneous interpretation.

Nitrites from nitrates.— Nitrates reduced.

Strain No. 191 was the only one that did not reduce nitrates to nitrites. The starch-K[illegible]-sulphuric-acid test recommended by Smith ('05) was employed.

Ammonia production.—Mod[illegible]erate.

Standard nitrate broth was used as a culture medium. Duplicate tubes were inoculated, one of which was sealed with paraffin. When this was tested at the end of the tenth day, using Nessler's solution, a yellow coloration appeared in all tubes except controls. The color was slightly deeper in the sealed tubes.

Fermentation.—Acid and small [illegible]olumes of gas from dextrose, lactose, sucrose, maltose, and mannite. No acid or gas from glycerin, dextrin, and potato starch. Moderate growth in closed [illegible]rm of fermentation tubes, from which dissolved oxygen is driven off, in presence of dextrose, lactose, sucrose, [illegible]naltose. No growth at first, feeble growth later in closed arm under similar conditions, in presence of glycerin, dextrin, and potato starch. Quantitative consumption of common hexose [illegible]ugars and of [illegible]rose, lactose, and maltose. No hydrolysis of dextrin and potato starch.

As was stated in an earlier paragraph, the several students of the blackleg bacillus differ in their published observations and conclusions as to the gas- and acid-producing capacity of different isolations of this parasite. This fact is clearly shown in the tabular summary presented herewith (table VI).

The results of the writer's fermentation studies are briefly summarized in table IV. The data presented here lead to the conclusion that the several strains of the organism studied (except No. 191) are similar and that all produce small quantities of gas and considerable acid from dextrose, lactose, and sucrose, but no acid or gas from glycerin or potato starch.

In the course of his studies the writer determined that some of the strains at hand produced gas and acid from fructose, galactose, and maltose as well (compare with results of quantitative experiments on carbohydrate consumption).

Among other things, it was determined that the gas-producing function of the blackleg bacillus varies, depending upon (1) oxygen relations, (2) the length of time cultivated in the presence of a sugar, (3) the composition of the culture medium, etc., more than upon the particular strain or strains of the organisms under observation. In one experiment (Exp. "A", table V) only the minutest volumes of gas collected in the closed arm of 3 of the tubes (lactose) out of a total of 90 inoculated (30 of lactose alone). In this set 1 per cent of the carbohydrate was added to Dunham's solution (the reaction was not adjusted) and distributed in fermentation tubes. The tubes were heated in streaming steam until the air dissolved in the solution had been expanded and driven out. The bubble which collected in the top of the closed arm of the tubes was carefully tilted off before the set was autoclaved. Small bubbles found in the closed arm of 2 of the tubes upon removal from the autoclave were tilted off. Invigorated cultures were used for inoculation, and this was accomplished in the usual manner as soon as the culture medium was cooled. The cultures were incubated at 27–28° C. A feeble to moderate growth developed in the closed arm of all tubes containing glucose, lactose, and sucrose, also in tubes under observation containing fructose, galactose, maltose, but not in closed arms of tubes containing glycerin, dextrin, and potato starch. Under the circumstances it may be concluded that the strains of the blackleg bacillus under observation grow anaerobically only when they obtain sufficient

TABLE IV

PHYSIOLOGY (FERMENTATION)

Culture No.	Medium containing Dunham's solution and:															Controls
	Dextrose			Lactose			Sucrose			Glycerin			Potato starch			
	Gas	Reaction*		Gas	Reaction		Gas	Reaction		Gas	Reaction		Gas.	Reaction		Gas
		1 dy.	5 dys.		1 dy.	5 dys.		1 dy.	5 dys.		1 dy.	5 dys.		1 dy.	5 dys.	
160.1	+	+4	+ 8	+	+2	+7	+	+3	+ 8	0		0	0		−1	0
170.3	+		+10	+		+6	+		+10	0		0	0		0	0
180.2	+		+ 7	+		+8	+		+ 9	0		0	0		0	0
183.2	+		+ 8	+		+7	+		+ 6	0		0	0		0	0
187B.1	+	+4	+10	+	+4	+7	+	+5	+ 9	0	−1	1	0		0	0
191	0		− 8	0		−6	0		− 7	0			0			0
194	+		+ 8	+		+5	+		+ 6	0		0	0		0	0
195	+	+3	+ 8	+	+1	+7	+	+3	+ 5	0	−2	−2	0	−1	−1	0
196	+	+4	+12	+	+4	+8	+	+5	+11	0	−1	−1.5	0	0	−2	0
197	+	+5	+10	+	+1	+7	+	+3	Lost	0	−1	−1	0		−0.5	0
198	+		+ 8	+		+9	+		+9	0		0	0		−0.5	0
201	+	+8	+10	+	+4	+6	+	+4	+9	0	−1	−1	0	+1	0	0

* Reaction data represent gain (+) or loss (−) in degrees on Fuller's scale, over controls, after time intervals specified.

TABLE V

GAS PRODUCTION

Cult No.	Exp. A			Exp. B			Exp. C		
	Dex.	Lac.	Suc.	Dex.	Lac.	Suc.	Dex.	Lac.	Suc.
160.1	0	0	0	0	+	+	+	+	+
170.3	0	0	0	0	+	+	+	+	+
180.2	0	+	0	+	+	+	+	+	+
183.2	0	0	0	+	+	+	+	+	+
187B.1	0	+	0	+	+	+	+	+	+
191	0	0	0	*	*	*	*	*	*
194	0	0	0	+	+	+	+	+	+
195	0	0	0	+	+	+	+	+	+
196	0	0	0	0	+	+	+	+	+
197	0	0	0	+	+	+	+	+	+
198	0	+	0	+	+	+	+	+	+
201	0	0	0	+	+	+	+	+	+

* No data.

TABLE VI

DATA ON CARBOHYDRATE REACTIONS

Investigator	Dextrose		Lactose		Saccharose		Glycerin		Diastatic action
	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	
van Hall	+	+	0	+	+	+	0	0	0
Appel	*	*	*	*	+	*	*	*	*
Smith	0	+	+	+	0	+	*	*	*
Harrison	0	+	+	+	0	+	0	+	+
Pethy. & Murphy	+	+	+	+	+	+	0	0	+
Morse	+	+	+	+	+	+	0	+	+
Paine	+	+	+	+	+	+	*	*	+
Shapov. & Edson	+	+	+	+	+	+	0	0	0

* No data.

oxygen for metabolic processes from oxygen-containing substances which they can break down. It may be further concluded that the gas production is inhibited by the lack of free O_2.

In another set of tests (Exp. "B," table v) the same strains were employed as before, being cultivated in plain peptone water to which 1 per cent of the carbohydrate was added. The reaction was not adjusted. The Board of Health type of fermentation tube was employed, and sterilization was accomplished in the autoclave. After autoclaving, the tubes were set aside for a few days. Just before inoculation, the medium in each was thoroughly mixed by tilting the tube, but no free bubbles of air

were left in the closed arm. Inoculation was made in the usual manner, using invigorated strains. The cultures were kept at room temperature, 18–22° C. Gas production took place in all but 3 of the cultures (Exp. "B", table v) as was evidenced by the accumulation of from 1 to 8 or 9 per cent of a gas in the closed arm. Only very slight increases in the volume of the gas present occurred after the end of the first week. During the first 4 to 6 days there was just about as much growth in the closed arm of the tubes as in the open arm.

Still another test of the gas-producing capacity of the black-leg strains was made, using agar shake cultures. Small amounts of gas were produced by all the strains tested (Exp. "C", table v), as was evidenced by the development of a greater or less number of bubbles in the medium.

The appearance of gas bubbles in 1-day-old glucose agar slant cultures suggested the use of this type of test. A 0.75 per cent meat extract agar was used. The reaction of this medium was not adjusted. To this was added 1 per cent of the carbohydrate to be investigated. The medium was melted, cooled to 42° C., and inoculated with 3-mm. loops of invigorated broth cultures. The inoculum was thoroughly mixed and the medium aerated by a vigorous rolling between the hands. The cultures thus made were set aside in a vertical position to cool and were incubated at 27–28° C. In this type of culture it was found that there was little increase in the volume of gas produced later than the second day after inoculation, as evidenced by the number and size of the bubbles.

In following up this line of experimental work it was later determined that when agar shake cultures were sealed with paraffin a somewhat greater volume of gas was demonstrable, as evidenced by the increased number and size of the bubbles in the medium. What is even more significant: It was found that the cultivation of a particular strain (or strains) in the presence of a certain carbohydrate increased its powers to ferment that carbohydrate with the production of gas. In this way certain strains which at first produced gas very weakly, if at all, were "trained up" to ferment the particular carbohydrate in question more vigorously, as was evidenced by the production of larger and larger volumes of gas.

Diastatic action.—Absent.

The writer's conclusion is based upon the results of carefully conducted quantitative determinations of carbohydrate hydrolysis by certain of the blackleg strains at hand. In this phase of the work the hydrolysis of potato starch, as well as other carbohydrates, was investigated. No evidence was found of the slightest hydrolysis of potato starch by the blackleg bacillus after an interval of 6 days. The ordinary tests for diastatic action were made, using a nutrient agar starch jelly, and these yielded no evidence of diastatic action. These results are not in accord with those of most investigators (table VI).

Active acidity and titratable acidity of culture solutions.—While the greater part of the experimental work reported upon here was done in 1916 and 1917, the data presented in this section were obtained in May, 1922. Experiments were made in order to determine the production of acid, as shown by the determination of H-ion concentration, as well as by the relative amount of titratable acid produced by *Bacillus atrosepticus* when cultivated in the presence of certain sugars. Subcultures of strain No. 196 were employed in these experiments.

To a nutrient broth made as before 1.0 per cent of the following sugars was added: (a) glucose ["Difco"], (b) sucrose [Merck's highest purity], (c) lactose [Merck's].

Cultures and methods.—Comparatively large-volume cultures were employed, 500 cc. of each of the sugar broths being placed in each of 3 Florence flasks of 1 liter capacity. Sterilization was accomplished in the autoclave at 15 pounds pressure for 15 minutes. In order to avoid the lag phase of growth (Chambers, '20) a rejuvenated broth culture 7 hours old was used for inoculating the sugar broth employed.

The H-ion concentration of each culture medium was determined at the time of inoculating the sugar broths and thereafter as indicated (table VII). The colorimetric method of Clark and Lubs ('17) was employed. The production of titratable acid was determined in the usual manner, using 0.507 NaOH and phenolphthalein, and the amount of titratable acid was expressed in degrees on Fuller's scale where "+10"=0.1 of 1 per cent normal HCl.

Buffer index.—Following the method of Brown ('21) the buffer index of the medium was determined in order to provide

additional data for comparison if need should arise. All 3 sugar broths employed were found to have a buffer index of 1.5 where "BI" is the sum of the reserve alkalinity and the reserve acidity, each value being expressed in per cent normal alkali or acid.

TABLE VII

H-ION CONCENTRATION AND TITRATABLE ACIDITY FOR STRAIN NO. 196

Medium	Cultures									
	Controls		1 day		2 days		5 days		11 days	
	P_H	Titr. acid.	P_H	Titr. acid.	P_H	Titr. acid.	P_H	Titr. acid.	P_H	Titr. acid.
Glucose.....	6.0	+16	5.4	+20	5.2	+22	5.0	+25	5.0	Not determined
Sucrose.....	6.0	+16	5.4	+21	5.0	+24	5.0	+25	5.0	Not determined
Lactose.....	6.0	+16	5.6	+20	5.2	+21	5.2	+25	5.1	Not determined

From table VII it may be noted at a glance that (1) some acidity is developed by *Bacillus atrosepticus* when grown in the presence of certain sugars, and (2) essentially equivalent amounts of acid are developed in the presence of all 3 sugars employed.

Attention is also directed to the fact that the major increase in H-ion concentration took place during the first 2 days, and it is believed (see Chambers, '20) that a true acidity of about P_H 5 inhibits further growth and reproduction of the organism.

SUMMARY OF COMPARATIVE STUDIES OF CAUSAL ORGANISMS

The salient characteristics of the several strains of the blackleg bacillus studied by the writer are brought together in compact form below. The scheme adopted is based upon the Descriptive Chart indorsed by the American Society of Bacteriologists in December, 1920. The digits used to designate the several "primary" and "secondary" characteristics of the strains in question may be translated in detail by reference to the foregoing text, and to the "brief characterization" column upon the descriptive chart above mentioned. The data below indicate the fact that there is very little, if any, difference between the organisms studied by the writer, except No. 191, which aside from being non-pathogenic to the potato, differs from the others in many important respects.

BRIEF CHARACTERIZATION

160.1	Montana strain	53*2–32120–2111–2222– *211–22–121
170.3	Montana strain	53 2–32120–2111–2222– 211–22–121
180.2	Montana strain	53 2–32120–2111–2222– 211–22–121
183.2	Montana strain	53 2–32120–2111–2222– 211–22–121
187B.1	Montana strain	53 2–32120–2111–2222– 211–22–121
191.	*B. phytophthorus* "as rec'd. from Appel."	53 –51230– 333–1122– 322–33–322
194.	Maine strain Morse's "SE"	53 2–32120– 111–2222– 211–22–121
195.	Maine strain Morse's "IIP"	53 2–32120–2111–2222– 211–22–121
196.	*B. solanisaprus* Harrison	53 2–32120–2111–2222– 211–22–121
197.	*B. atrosepticus* van Hall	53 2–32120–2111–2222– 211–22–121
198.	*B. melanogenes* Pethy. & Murphy	53 2–32120–2111–2222– 211–22–121
201.	*B. phytophthorus* Appel	53 2–32120–2111–2222– 211–22–121

While all differences, great or small, have been recorded, it is significant that these differences, except in the case of No. 191, are insufficient to appear in the brief characterization of each strain set down above. Such variations as may exist are, it seems to me, wholly insignificant and do not justify even varietal characterization. Within the limits of his comparative studies Morse ('17) reached similar conclusions. Among his cultures were some of the same strains used by the writer, namely, Nos. 194, 195, 196, 197, and 198.

RELATIONSHIPS AND NOMENCLATURE

Differences between the 4 "species" of the blackleg bacillus as originally described may be noted by referring to the original descriptions or to abstracts prepared by the writer (see p. 17 et seq.).

The index numbers brought together below assist in visualizing the small differences upon which certain of these "species" were established.

B. atrosepticus van Hall (van Hall, '02).....5312-32120-2121
B. phytophthorus Appel (Appel, '03) .....5?1?-32120-???1
B. phytophthorus Appel (Smith, '10).....5312-32120-?212
B. solanisaprus Harrison (Harrison, '07) 5312-32120-1212
B. melanogenes Pethy. & Murphy (Pethybridge and Murphy, '10) 5312-32110-1111

* Omission of figure indicates item not determined.

Reference to the literature discloses the fact that Appel ('02, '02a) succeeded at an early date in isolating a bacterial organism which he believed to be the cause of the potato disease in question. The motive which prompted him to publish brief notes (Appel, '02, '02a) on the disease, and at the same time to propose a name (only) for the causal organism, must be left for conjecture. The fact remains that he (Appel, '03) did not publish a full description of either the organism or the disease until about a year after van Hall's ('02) dissertation was available to science.

Pethybridge and Murphy ('11) must have recognized that their species was very similar to van Hall's and Appel's, for in regard to "*B. phytophthorus*" and "*B. melanogenes*" they state: . . . "the two organisms, if not identical, are at any rate closely allied; and it is perhaps with some reluctance that we regard it as a distinct species and suggest the name *Bacillus melanogenes* for it." Relative to *B. atrosepticus* van Hall, these authors say that it has some points in common with their species, but differs by "occurring chiefly as isolated individuals, whereas ours is more frequently found in pairs. The former is also decidedly smaller in size, in spite of variations in both cases, and its action on milk appears to be different from that found in our case. *We were unfortunately unable to obtain a copy of the detailed character of B. atrosepticus* [italics not in original] before our own work was concluded."

Harrison ('07) states that "the symptoms of the Ontario disease somewhat resemble those described by Appel for the 'Schwarzbeinigkeit,' a disease which seems rather widespread in Germany." Further than this, the only comparisons made by him appear in a little display chart on page 592 of his paper. In this exhibit certain of the morphological and biological characteristics of "*B. phytophthorus*", "*B. atrosepticus,*" and "*B. solanisaprus*" are compared. The differences brought out are, it seems to me, more apparent than real, as shown by the index numbers compiled (see p. 41).

As was pointed out in an earlier paragraph, Appel's name "*Bacillus phytophthorus*" appears as a *nomen nudum* in a brief paper published by him about two months previous to the appearance of van Hall's "*Bacillus atrosepticus.*" Furthermore, van Hall's dissertation embodies the earliest published description of the potato blackleg disease which is sufficiently definite

and detailed to enable one to identify the disease ascribed to the bacterium named and described as the cause.

For a number of reasons Smith ('20) believes it best to retain Appel's name, especially as van Hall made very few inoculations under natural conditions, and further, because he says of his organism: "On artificial media the parasite loses its virulence very quickly." This contention loses weight in the light of a similar statement made by Smith on a preceding page (see Smith, '20, page 263), as follows: "It is common belief (*of German origin*) [italics not in original] that the organism loses virulence readily." The writer feels that Smith's further objections to the use of van Hall's name are largely refuted by the data and facts previously presented.

It was inevitable that questions involving nomenclature should have appeared in this paper and a serious attempt has been made to give all names most careful consideration. On the grounds of priority as well as for other reasons brought out above the writer believes that *Bacillus atrosepticus*[1] van Hall should stand.

REVISED DESCRIPTION OF BACILLUS ATROSEPTICUS VAN HALL

Index No. 5312-32120-2111.[2]

Microscopic features.—The potato blackleg bacillus is a small non-sporiferous, Gram-negative rod, having an average diameter of about 0.6 μ and a length (1.5 μ) slightly exceeding twice its diameter. The organism is actively motile by means of a few peritrichic flagella. No capsule is demonstrable by the ordinary methods now in use.

Physiological characteristics and cultural features.—The organism is non-chromogenic in agar, gelatin, nutrient broth, and other common media. On agar slants growth is moderately abundant. The surface is smooth and the luster is glistening. Agar colonies are small, round to somewhat irregular in form, and, under a magnification of 50 diameters, they appear to be granular in structure.

Gelatin is liquefied, the action being visible on the second day, if not before. Colonies on gelatin plates are white, round, and noticeably larger than those which develop on agar.

[1]According to a recently suggested outline of bacterial classification (Winslow et al, '20) the name would become *Erwinia atrosepticus* nov. comb.

[2]See Descriptive Chart indorsed by Soc. of Am. Bacteriologists, Dec. 30, 1920.

Nutrient broth is promptly clouded, the medium becoming slightly turbid after a few days. The characteristic surface growth is a ring, though in some broths a light pellicle may develop.

Milk is promptly coagulated, the amount of titratable acid produced by the organism increasing steadily for several days. A slow peptonization of the curd begins on the second or third day.

In Cohn's solution there is no growth. In Uschinsky's solution the growth is copious, but the fluid does not become viscid.

No indol is demonstrable by the Ehrlich test, in either young or old cultures grown in peptone water made with (1) Witte peptone, or (2) Bacto peptone. Nitrates in nitrate broth are reduced to nitrites without the formation of gas. Ammonia production is feeble to moderate.

Carbohydrate reactions.—Acid and small volumes of gas are produced from dextrose, galactose, sucrose, maltose, and mannite. The gas-producing capacity is not particularly characteristic. No acid and no gas are produced from glycerin, dextrin, and potato starch. Quantitative consumption of the common hexose sugars, glucose, fructose, and galactose, has been demonstrated. Likewise, the organism consumes sucrose, lactose, and maltose. A sugar concentration of 0.25 per cent is ample. Diastatic action is absent, both in respect to starch and dextrin.

Enzymes.—This organism secretes several carbohydrate enzymes, as is shown by its action on as many different saccharides; cytase also is probably produced.

The optimum temperature for growth is about 26° C., with no growth at 37.5° C. The organism withstands considerable extremes of cold, being found viable in soil cultures exposed for 24 hours to temperatures ranging from —6.7° to —28.2°C.

The blackleg bacillus is quite resistant to drying and remains viable for long periods of time on plain beef agar.

Virulent strains produce a necrosis of the stem and tubers of the potato. Parenchymatous tissue is almost exclusively affected, and a blackening of the diseased tissues is characteristic.

III. Carbohydrate Utilization by Strains of the Blackleg Bacillus and Other Microorganisms

In spite of the fact that the strains of the blackleg bacillus employed by the writer in earlier studies showed no signs of diastatic action on potato starch jelly, it was nevertheless somewhat difficult to conceive of a virulent parasite of the potato totally lacking the power of hydrolyzing starch. This thought, together with a rather extensive knowledge of the gas- and acid-producing capacity of this bacillus, suggested the desirability of investigating these relations in a quantitative manner. The writer had employed Shaffer's ('14) modification of the volumetric method of Bertrand for the quantitative estimation of reducing sugars in the presence of proteins and albumins. Familiarity with this method led me to conclude that with its aid reliable data could be obtained concerning the quantitative consumption of certain sugars and starch by the blackleg bacillus as well as by other microorganisms with which it appeared. Very little, if anything, had been published upon the quantitative consumption of carbohydrates. Accordingly, plans were made to investigate quantitatively these relations in certain of the strains of the potato blackleg bacillus as well as in other species of bacteria. Some points of interest in this connection have, however, recently been contributed by Besson, Ranque, and Senez ('19). Working with *Bacillus coli* in nutrient broth containing varying amounts of dextrose, they found that when less than 0.4 per cent dextrose was furnished all the sugar was removed in 24 hours.

MATERIALS AND METHODS

After numerous experiments a method was developed whereby reliable data were obtained. The work of Shaffer and Hartmann ('21) on the iodometric determination of copper and its use in sugar analysis had not appeared at the time the writer performed the experiments reported herewith. Undoubtedly, the newer methods would have greatly facilitated prosecution of this phase of the work, but it is doubtful if more accurate results could have been obtained.

Certain strains of the blackleg bacillus used in the preceding studies were selected for investigation and comparison in this connection, i. e., Nos. 160.1, 180.2, 183.2, 187B.1, 195, 197, 198, 201. Pure cultures of the following were also tested: *Bacillus coli*

communis, B. vulgaris, and a species of yeast isolated from a compressed yeast cake.

The basic culture solution used throughout was a Dunham's solution to which 1 per cent of the following test substances (Merck's, except starch) was added: (1) glucose (H. P.), (2) fructose (cr.), (3) galactose (powder), (4) saccharose (H. P.), (5) lactose (H. P. cr.), (6) maltose (powder), (7) dextrin (H. P. powder), and (8) potato starch (Heil's).

The nutrient solutions used were not adjusted by the addition of acid or alkali. As a matter of fact, it was determined in a preliminary experiment that if small amounts of acid (HCl) were added to adjust the reaction a considerable hydrolysis of certain disaccharide sugars resulted during the process of sterilization, regardless of whether the latter was accomplished in the autoclave or in the Arnold sterilizer (table IX). The Dunham's solution employed as a basis of the nutrient solutions used was found to be P_H 7.0, approximately. The P_H of the several sugar broths employed was not determined, but it is unlikely that they differed markedly in H-ion concentration from the Dunham's solution.

Invigorated cultures of the several microorganisms at hand were employed. Ten-cc. volumes of the media were measured into special culture tubes, called wasp tubes (see pl. 2, fig. 5). (The wasp tube was suggested to me by Dr. G. W. Freiberg, formerly of this laboratory, and has greatly facilitated and expedited my work.) These were plugged in the usual manner and sterilization was accomplished in the autoclave (see table IX). Duplicate cultures were made, and a number of controls were carried along with each series of cultures. All cultures were incubated at 28° C. for 6 days, as it was found that there was no point in carrying the cultures for a longer period. Chambers ('20) has recently contributed a convincing explanation of why evidences of the metabolic activities fail after a few days in certain sugar-broth cultures.

Shaffer's ('14) method for the quantitative estimation of reducing sugars was used with slight modifications. This development of the permanganate titration method of Bertrand is of special value to workers in plant physiology because it enables one quickly and accurately to determine the amount of sugar as low as 2 mgms. The copper-reducing value of the culture

medium in which the microorganisms in question were grown was determined by this method, as was the copper-reducing value of control portions. Differences were carefully noted and carbohydrate utilization estimated by reference to Shaffer's table of copper-glucose equivalents, having first determined by calculation that 1 cc. of the N/20 permanganate is equivalent to 3.18 mgms. copper. For the technique of the complete method see under "Technique."

According to Plimmer ('15) pure lactose reduces Fehling's solution 71 per cent as strongly as glucose; pure maltose 64 per cent as strongly as glucose. These figures were employed in making estimates of the amounts of these disaccharides consumed by the microorganisms under consideration. It was assumed in this work that the fructose and galactose used reduced Fehling's quite as strongly as glucose. Certain dextrins reduce Fehling's slightly, but the product which I employed did not; nor did the saccharose and potato starch. Where it became necessary to hydrolyze these last-named substances in order to determine whether there had been utilization of the substance as such, the following methods were used:

Sucrose.—Davis and Daish ('13) employed citric acid in preference to a mineral acid for inverting cane sugar. In dealing with plant extracts, in which there was an accumulation of sodium acetate, they employed 10 per cent citric acid.

Method.—(1) Make solution faintly acid to methyl orange by addition of a few drops of concentrated H_2SO_4; (2) add 5 per cent by weight of citric acid; (3) autoclave for 15 minutes at 15 pounds pressure, cool, and neutralize to phenolphthalein with NaOH.

Starch and dextrin. Method.—(1) To a 1 per cent starch suspension (10 cc.) add 0.3–0.5 cc. conc. HCl and 10 cc. distilled water; (2) heat in autoclave for 15 minutes at 15 pounds pressure; (3) cool, neutralize to phenolphthalein with NaOH, and estimate as glucose. The amount of glucose times 0.9 equals the weight of starch hydrolyzed.

Maltose and lactose. Method.—(1) To 10 cc. of a 1 per cent solution of the sugar add 0.3–0.5 cc. conc. HCl (sp. gr. 1.16) and 10–15 cc. distilled water; (2) heat in autoclave for 15 minutes at 15 pounds, cool and neutralize to phenolphthalein by addition of NaOH; (3) dilute and estimate as glucose.

Complete reduction of these sugars is difficult, if not impossible, to accomplish by boiling for 60 to 90 minutes. Ordinarily, inversion is complete to the extent of 96–97 per cent. Heating for a longer time will accomplish more inversion, but is offset by an increasing destruction of the resulting monosaccharides.

A trial of the method developed by Ling and Rendle ('05) showed that this could not be used because of (1) a clouding of the culture solution immediately upon addition of the Fehling's, making it practically impossible to observe discharge of blue color upon nearing the end point, and (2) the CuO_2 does not settle readily. The trouble is probably due largely to the amino acids of the peptone in the Dunham's solution employed (cf. Davis and Daish, '13).

REAGENTS AND SOLUTIONS

Fehling's solution.—It was found best to use the Soxhlet-Fehling solution, preliminary tests having demonstrated that the solution as modified by Allihn contained too much alkali, when made up with NaOH at the rate of 178 gms. per liter.

Cuprous oxide solvent.—The ferric sulphate-sulphuric acid-cuprous oxide solvent recommended by Shaffer ('14) was employed. This solvent contains 10 per cent ferric sulphate, $Fe_2(SO_4)_3$, in 25 per cent sulphuric acid. It is a very active solvent, and care must be taken in watching for the end point. To be efficacious it should be made up by mixing equal parts of a 20 per cent aqueous ferric sulphate and 50 per cent sulphuric acid. The ferric sulphate should be dissolved in hot water, filtered while hot, and the warm acid mixed with the warm ferric sulphate solution. Just enough permanganate should be added to oxidize any ferrous salt which may be present.

Potassium permanganate solution.—The potassium permanganate used was made up according to Olsen ('10). Freshly prepared and carefully standardized N/20 permanganate was employed for titration. One cc. of N/20 permanganate is equivalent to 3.18 mgms. of copper.

TECHNIQUE

The determination of reducing sugars.—The following technique was followed in determining amounts of reducing sugars in carbohydrate broth cultures of bacteria. Certain slight modi-

fications of Shaffer's ('13) original method appear, but these are largely omissions of certain steps which were determined by preliminary experimentation to be unnecessary. For instance, acetic acid and colloidal iron were not used in the process because it was determined that the small amounts of albuminous material present in the cultures did not interfere with the determination of the reducing sugars present. The precautions emphasized by Shaffer were observed throughout. The cuprous oxide was titrated immediately upon being dissolved and without removal from the centrifuge tube in which it was precipitated and thrown down. In order to avoid breakage of the centrifuge tubes when these were plunged in the water bath circular wire baskets (pl. 2, fig. 5) having wooden bottoms and tops, with holes for 2 centrifuge tubes, were used. No oxidizable substance other than the sugar was allowed to get into the solution, and the cuprous oxide solvent employed was made from chemically pure substances in order to avoid presence of ferrous iron. This was further assured by adding a trace of permanganate.

Procedure.—The procedure outlined in the steps given below was followed in carrying out all experiments in this phase of the work:

(1) A large volume of Dunham's solution was made up, autoclaved, and then filtered through paper.

(2) To separate portions of this solution, 1 per cent of the test substances (see list p. 46) was added and dissolved.

(3) Exactly 10 cc. of each of the 8 nutrient solutions thus prepared were placed in each of 10 special culture tubes ("wasp" tubes, pl. 2, fig. 5). These tubes were large enough to permit dilution of the culture to exactly 60 cc. This was made possible by carefully standardizing each tube and permanently etching the 60-cc. mark on the slender neck. Had these tubes been drawn out and graduated to hold 50 cc., calculations would have been simplified.

(4) The tubes were plugged with cotton in the usual manner and the contents sterilized in the autoclave by exposing for 15 minutes at 15 pounds pressure. One of the preliminary experiments performed showed that this method, if carefully controlled, did not hydrolyze the di- and polysaccharides used (table IX).

(5) Each of the 8 media was inoculated with a loopful of an invigorated culture of the organism to be tested for its ability to hydrolyze the test substances employed. Two uninoculated "control" tubes of each sugar broth were carried along, incubated, and tested in identically the same manner as the inoculated cultures in each set.

(6) The sets thus made ready were incubated at 27–28°C. for 6 days, preliminary tests having shown that there was no point in incubating the cultures for more than 5 or 6 days (cf. Chambers,'20).

(7) The culture medium in each "wasp" tube was next diluted 6 times by adding distilled water to the 60-cc. mark. The contents of each was thoroughly mixed.

(8) Next, exactly 10 cc. of the diluted culture medium were removed and placed in a 50-cc. lipped centrifuge tube (pl. 2, fig. 5), which was marked to correspond to the culture tube from which the medium came. These 10-cc. volumes, then, represented 1/6 of the original culture solution, i.e. 1.666 cc. of the original 10; and if the 10 cc. of the culture solution contained 0.1 gm. of a carbohydrate a 10-cc. sample from the diluted culture should contain 0.0166 gm. of the substance provided none had been consumed.

Each tube in the set was handled in exactly the same way, the same pipette being used throughout.

(9) Ten cc. of freshly mixed Soxhlet-Fehling's solution were promptly added to the 10-cc. volumes in the centrifuge tubes.

(10) All were quickly placed in the special baskets and immersed in a water bath (pl. 2, fig. 5) where they were exposed at the boiling point for exactly 10 minutes.

(11) Upon removal from the bath the tubes were nearly filled with distilled water, pairs balanced, and centrifuged[1] at a moderate speed for 3–4 minutes.

(12) Upon removal from the centrifuge the Fehling's was cautiously decanted over a white dish, the cuprous oxide at the bottom being disturbed as little as possible, for fear of loss.

(13) This having been done each tube was again filled with distilled water in order to wash the precipitate, pairs balanced, and again centrifuged for about 4 minutes.

(14) When finally removed from the centrifuge the wash water was decanted as completely as possible without disturbing the cuprous oxide in the bottom (if any was present).

(15) The cuprous oxide was then promptly dissolved in as small a volume of the ferrous sulphate-sulphuric acid solvent as possible.

(16) The copper was then titrated immediately against N/20 potassium permanganate. No attempt was made to remove the dissolved cuprous oxide from the centrifuge tube, the actual amount of copper present being determined by titrating directly into the tube.

(17) Using the figures thus obtained and remembering that the 10-cc. sample titrated represents 1.666 cc. of the original culture medium,

[1] The No. 1 centrifuge of the International Instrument Co. was used.

the actual amount of sugar (as glucose) left was calculated by reference to Shaffer's ('14) table of copper-glucose values. In order to determine consumption of the non-reducing carbohydrates in use, i.e., sucrose, dextrin, and starch, it was necessary to accomplish inversion by heating in the presence of an acid (see "Methods," p. 47, et. seq). This having been accomplished, the procedure was exactly as outlined above.

The control tubes containing the several carbohydrate broths were handled in exactly the same manner as the cultures, except for inoculation, and were a component of each experiment.

EXPERIMENTAL DATA

The data relative to carbohydrate utilization are presented in tables IX–XIII. Where practicable the amounts of the test substance consumed are given in mgms. of the carbohydrate supplied. The most complete data are, for obvious reasons, given in terms of mgms. of glucose. The writer believes that the figures are reliable to the extent of showing relative differences in carbohydrate utilization as well as the amounts of the test substances consumed by each of the several species and strains of the microorganisms employed.

A slight error in the sugar data presented may have developed, due to the fact that it was necessary to make interpolations between figures available in tables IX–XIII, in order to determine the sugar equivalent of the copper values obtained from my own permanganate titration data. The following figures and statements are presented to illustrate the methods and the calculations employed in obtaining the sugar values presented in the above-mentioned tables. The specific case of *Bacillus coli* in the presence of glucose is selected for this illustration. The 10-cc. sample of the culture taken for treatment with Fehling's and subsequent titration represented (as in all other cases) 1.666 cc. of the original 10-cc. sugar broth in which the organism was cultivated. It was not necessary to account for possible loss due to evaporation, since the cultures were made up to 60 cc. before sampling.

Having determined the number of cc. of permanganate for 1 cc. of the original culture solution as 4.15, then the number of mgms. of copper is represented by 13.2, determined by multiplying 4.15 by the factor 3.18 (it was previously determined that 1 cc. of N/20 permanganate is equivalent to 3.18 mgms. copper).

Next, the glucose equivalent of the 13.2 mgms. copper is estimated by interpolating in Shaffer's table of copper-glucose equivalents, and finally, the amount of glucose consumed or used by the organism is found by determining the difference between the amounts of glucose recovered in the control and in the culture.

Where amounts of sucrose are given they were determined as follows:

$$96 : 100 :: A : X,$$

where the reducing ratio

$$\frac{\text{Glucose}}{\text{Invert sugar}} = 0.96,$$

and A is the invert sugar ("glucose") value obtained by titration and calculation, and X equals the true glucose value.

Then:

$$360 : 342 :: X : Y,$$

where 360 is the molecular weight of 2 molecules of $C_6H_{12}O_6$ and 342 is the molecular weight of $C_{12}H_{22}O_{11}$. Then, where X equals true glucose value determined above, Y equals sucrose consumed. Fructose and galactose consumption were calculated from the "glucose" values obtained, using the reducing ratios of these sugars as given by Browne ('12):

$$\text{Ratio } \frac{\text{Glucose}}{\text{Fructose}} = 0.92,$$

$$\text{Ratio } \frac{\text{Glucose}}{\text{Galactose}} = 0.90.$$

Consumption of lactose and maltose is directly determinable, since both these sugars are reducing sugars. Consumption of lactose, maltose, dextrin, and starch, as glucose, was determined by hydrolyzing portions of both control and culture solutions (see "Methods," p. 47). For well-known reasons no attempt was made to calculate consumption of dextrin and starch as such, nor was it thought to be worth while to convert the galactose-glucose value obtained by titrating the hydrolyzed lactose into glucose. It should be borne in mind that the glucose values given for dextrin and starch were derived by multiplying the glucose value obtained by titrating the hydrolyzed product by 0.9. Without doubt the figures in the tables representing consump-

tion are slightly erroneous, since, as is well known, more or less destruction of hexose sugar takes place in the process of hydrolyzing carbohydrates by heating in the presence of an acid. It may be recalled, too, that variability in the reducing power of lactose and of maltose is a characteristic of these disaccharides. According to Browne ('12, p. 402), succeeding portions of lactose and maltose will vary in reducing power according to the amount of free alkali, time of boiling, etc. "This peculiarity of maltose and lactose" he says, "is explained by a slight hydrolysis of the sugar into monosaccharides of higher reducing power" during the process of heating. Browne believes that a slight inversion of this kind takes place to a greater or less extent with all higher saccharides (including sucrose) upon boiling with Fehling's solution.

These facts may account in part for the inconsistency of findings reported in the literature, to show that moist heat of moderate degree causes hydrolysis of certain higher disaccharides and other carbohydrates, such as dextrin and starch.

In carrying out a number of tests preliminary to the major work reported upon in connection with this phase of the investigation, one experiment is worthy of particular consideration,—that made to determine the effect of moist heat upon certain carbohydrates. The temperatures used were those encountered in sterilizing solutions in the autoclave and by the discontinuous method. Three different carbohydrate-containing solutions were made up: (1) distilled water with 0.5 per cent of the carbohydrates to be tested, (2) Dunham's solutions, acidulated by the addition of 0.4 per cent N/1 HCl, separate portions of which contained 1 per cent of the carbohydrates, (3) a plain Dunham's solution which contained 1 per cent of the carbohydrates being tested. Each of these lots was separated in 3 portions. The controls were not heated and were titrated immediately. The second set was autoclaved at 15 pounds (121.3° C.) for 15 minutes, the total length of time above 100° C. being 45 minutes. The third set was exposed in an Arnold steamer for intervals of 20 minutes at about 99.5° C. on 3 consecutive days. The findings appear in table IX, and it will be seen from the tabulated data that sterilization of certain saccharide broths was accomplished by either method without significant hydrolysis of the carbohydrates present when no acid (HCl) was added. Certain of the monosaccharides, as well as the disaccharides lac-

tose and maltose, dissolved in the Dunham's solution appeared to have been slightly altered during the process of sterilization in the Arnold sterilizer, but it seems doubtful if the changes which are indicated by the figures are significant even in the case of lactose and maltose, since, as has been pointed out, these sugars are characterized to a certain extent by a variability in reducing power.

On the other hand, reference to table IX emphasizes clearly the fact that a considerable hydrolysis of the di- and polysaccharides occurred in the acidulated broths exposed in the autoclave, and to a lesser extent in those exposed in the steamer. It also appears that sucrose is most markedly affected. According to my tests, lactose, maltose, and potato starch in slightly acidulated broth are not hydrolyzed by exposure to moist heat in the process of sterilization at 100° C.

The data given below are of interest when compared with the quantitative data presented in table IX. These data, furthermore, serve as a check on the other type of analysis.

TABLE VIII

RESULTS OF COLORIMETRIC TEST WITH IODINE.

Carbohydrate	Controls	Autoclave	Arnold Steamer
Dextrin	Magenta	No color	Very pale magenta
Potato starch	Blue	Magenta	Blue

In the other tables (IX-XIII) are presented data bearing on the quantitative consumption of chosen carbohydrates by a few microorganisms. Nine of the 12 cultures employed were representative strains of the blackleg bacillus and among the 9 were the 4 "species" of this bacillus, which, together with the other strains, were studied comparatively at an earlier date (see Part II). In addition to these, cultures of *Bacillus coli, B. vulgatus,* and a species of yeast (probably a species of *Saccharomyces*) were investigated. For the sake of facilitating comparisons the amounts of carbohydrate consumed, in terms of glucose, are assembled in table XIV.

DISCUSSION

The data presented in tables IX–XIV call for very little further commentary, but the action of strain numbers 195 and 196 (table XI and XII) in the presence of sucrose attract special

attention as being extraordinary for the bacillus represented. It would appear that these strains, unlike others of *Bacillus atrosepticus*, inverted cane sugar faster than the hexose by-products were used up. Also, it will be noted that these strains consumed nearly triple the average amount of sucrose used by the other blackleg strains. In this respect these cultures are comparable with *B. vulgatus*, which is the only other one of the 12 which consumes sucrose so strongly and, in addition, inverts this saccharide faster than it uses up the invert sugar. It is possible that these 2 cultures (Nos. 195 and 196) were contaminated during the course of these experiments. Neither attracted attention because of extraordinary cultural features at the time and so were not plated out.

Figures are presented in tables XI, XIII, and XIV which indicate a very slight consumption of dextrin by the blackleg strains number 187B.1 and 198. Also, it would appear that minute quantities of starch were used by strain No. 201 (table X), and it would seem that lactose was attacked by *B. vulgatus* as well as maltose by the yeast species (tables XIII and XIV). The writer is of the opinion, however, that these data in themselves are slightly misleading. It seems to him more than likely that they appear as the result of small discrepancies which are likely to develop in work of this nature and for reasons which have been stated above. It will be noted that the strain of *Bacillus coli* investigated did not hydrolyze sucrose nor consume it. *Bacillus vulgatus* as cultivated in these experiments did not utilize lactose nor did it use more than about a third as much galactose as glucose and fructose. Both these bacteria, it will be noted, attack with considerable avidity all the other carbohydrates employed. The yeast species used was unable to attack and use carbohydrates presented other than glucose and fructose. These sugars, however, were totally eliminated from the broth by this microorganism.

The several strains of *Bacillus atrosepticus* employed in these tests were quite alike in respect to their attack upon and use of the several carbohydrates presented. It appears likely that 15–20 per cent of the amount of the carbohydrates presented would have been sufficient to supply the strains employed, under the conditions of the experiments. The strains of the blackleg bacillus, furthermore, utilized sucrose, leaving no invert sugar behind except in the cases cited above of strains Nos. 195 and

TABLE IX

EFFECT OF METHOD OF STERILIZATION ON CARBOHYDRATES

Carbohydrates tested	0.5% in aqueous sol.					1% in Dunham's sol. +10 by HCl					1% in Dunham's sol. Not adjusted				
	Contrl.*	Autoclave		Arnold		Contrl.	Autoclave		Arnold		Contrl.	Autoclave		Arnold	
	Total recovery	Total recovery	Gain due to heat	Total recovery	Gain due to heat	Total recovery	Total recovery	Gain due to heat	Total recovery	Gain due to heat	Total recovery	Total recovery	Gain due to heat	Total recovery	Gain due to heat
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Glucose	4.7	4.7	0	4.7	0	10.2	10.2	0	10.2	0	10.6	10.5	0	10.6	0
Fructose	4.1	4.1	0	4.1	0	9.07	9.14	0.07	9.14	0.07	9.1	8.95	0	9.17	0.07
Galactose	4.4	4.1	0	4.1	0	9.26	9.25	0	9.27	0.01	9.15	9.2	0	9.2	0.05
Sucrose	0	0	0	0	0	0	11.5 (11.0)	11.5	11.3 (10.8)	11.3	0	0	0	0	0
Lactose	3.3	2.8	0	3.3	0	6.35	7.27	0.92	6.35	0	5.35	5.44	0.09	5.5	0.2
Maltose	2.32	2.31	0	2.31	0	4.6	6.07	2.67	4.55	0	3.9	3.89	0	4.18	0.28
Dextrin	0	0	0	0	0	0	1.44	1.3	0.85	0.76	0	0	0	0	0
Potato starch	0	0	0	0	0	0	1.04	0.9	0	0	0	0	0	0	0

* Not heated.

TABLE X

CARBOHYDRATE CONSUMPTION OF BACILLUS ATROSEPTICUS STRAINS

	Controls		No. 201				No. 197			
	Total recovery		Total recovery		Consumption		Total recovery		Consumption	
	Carb.*	Gluc.†	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Glucose	9.3	9.3	8.2	8.2	1.1	1.1	8.4	8.4	0.9	0.9
Fructose	8.4	9.2	7.3	8.0	1.1	1.2	7.4	8.0	1.0	1.2
Galactose	8.8	9.8	7.7	8.6	1.1	1.2	7.6	8.5	1.2	1.3
Sucrose	0	9.8 (9.4)‡	0	8.5 (8.2)‡	1.2	1.3	0	8.7 (8.4)‡	1.0	1.1
Lactose	5.8	9.8	4.7	7.1	1.1	1.9	4.7	7.0	1.1	2.0
Maltose	4.8	9.4	4.0	8.6	0.8	0.8	4.1	8.6	0.7	0.8
Dextrin	0	8.1	0	8.1	0	0	0	8.1	0	0
Potato starch	0	8.2	0	8.1	0	0.1	0	9.0	0	0

* Carbohydrate.
† Glucose.
‡ Invert sugar value in parenthesis.

TABLE XI

CARBOHYDRATE CONSUMPTION OF BACILLUS COLI AND B. ATROSEPTICUS STRAINS

	Controls		Bacillus coli				B. atrosepticus							
							No. 187B.1				No. 196			
	Total recovery		Total recovery		Consumption		Total recovery		Consumption		Total recovery		Consumption	
	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Glucose	9.5	9.5	6.0	6.0	3.5	3.5	8.3	8.3	1.2	1.2	7.8	7.8	1.7	1.7
Fructose	8.5	9.2	5.5	6.0	3.0	3.2	7.7	8.3	0.8	0.9	7.5	8.1	1.0	1.1
Galactose	8.7	9.7	5.4	6.0	3.3	3.7	7.8	8.7	0.9	1.0	7.3	8.1	1.4	1.6
Sucrose	0	9.7 (9.3)	0	9.7 (9.3)	0	0	0	8.7 (8.3)	0.9	1.0	2.2* (2.1)	6.0* (5.8)	3.5*	3.7*
Lactose	5.7	9.0	3.3	6.3	2.4	2.7	5.1	7.8	0.6	1.2	4.9	7.8	0.8	1.2
Maltose	4.5	9.4	3.2	6.2	1.3	3.2	4.2	8.7	0.3	0.7	3.2	7.0	1.3	1.4
Dextrin	0	8.2	0	6.8	+	1.4	0	8.0	0	0.2	0	8.2	0	0
Potato starch	0	8.1	0	6.0	+	2.1	0	8.1	0	0	0	8.2	0	0

* These figures seem to indicate an extraordinary sucrose consumption (but see pages 55 and 62). Certain of them indicate that the sucrose was inverted faster than used by the organism. See also No. 195, table XII.

TABLE XII

CARBOHYDRATE CONSUMPTION BY BACILLUS ATROSEPTICUS

	Controls		No. 195				No. 180.2				No. 160.1			
	Total recovery		Total recovery		Consumption		Total recovery		Consumption		Total recovery		Consumption	
	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Glucose	9.5	9.5	8.7	8.7	0.8	0.8	8.2	8.2	1.3	1.3	8.4	8.4	1.1	1.1
Fructose	8.7	9.5	7.6	8.3	1.1	1.2	7.4	8.0	1.3	1.5	7.7	8.4	1.0	1.1
Galactose	8.7	9.7	7.4	8.2	1.3	1.5	7.4	8.2	1.3	1.5	8.2	9.1	0.5	0.6
Sucrose	0	9.7 (9.3)	2.0* (1.9)	6.1 (5.9)	3.4	3.6*	0	7.5 (7.2)	2.1	2.2	0	8.7 (8.4)	0.9	1.0
Lactose	6.0	9.5	5.0	8.0	1.0	1.5	4.9	7.4	1.1	2.1	5.6	7.8	0.4	1.7
Maltose	4.5	9.2	4.4	8.8	0.1	0.4	4.0	8.0	0.5	1.2	3.9	8.6	0.6	0.6
Dextrin	0	8.1	0	8.1	0	0	0	8.1	0	0	0	8.1	0	0
Potato starch	0	8.1	0	8.1	0	0	0		0	0	0	8.1	0	0

* Cf. also No. 196 table XI.

TABLE XIII

CARBOHYDRATE CONSUMPTION BY BACILLUS VULGATUS, A YEAST, AND STRAINS OF B. ATROSEPTICUS

	Controls		Bacillus atrosepticus								Bacillus vulgatus				A yeast			
			No. 198				No. 183.2											
	Total recovery		Total recovery		Consumption		Total recovery		Consumption		Total recovery		Consumption		Total recovery		Consumption	
	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.	Carb.	Gluc.
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.
Glucose	9.4	9.4	8.4	8.4	1.0	1.0	8.2	8.2	1.2	1.2	5.4	5.4	4.0	4.0	0	0	All	All
Fructose	8.6	9.4	7.4	8.0	1.2	1.3	7.2	7.8	1.4	1.6	4.6	5.0	4.0	4.4	0	0	All	All
Galactose	8.7	9.7	7.7	8.5	1.0	1.2	7.8	8.6	0.9	1.1	7.4	8.2	1.3	1.5	9.1	10.0	0	0
Sucrose	0	9.6 (9.2)	0	8.3 (8.0)	1.2	1.3	0	8.2 (7.9)	1.3	1.4	6.0†	7.3 (7.0)	2.2	2.3	0	9.6 (9.2)	0	0
Lactose	5.9	9.0	5.0	7.4	.9	1.6	4.9	6.9	1.0	2.1	5.8	8.9	0.1	0.1	6.0	9.2	0	0
Maltose	4.5	9.3	4.9*	7.4†	?	1.9	4.1	8.5	0.4	.8	2.3	5.9	2.2	3.4	4.5	9.1	0	0.2
Dextrin	0	8.2	0	8.0	0	0.2	0	8.2	0	0	1.3†	4.9	0	3.3	0	8.2	0	0
Potato starch	0	8.1	0	8.2	0	0	0	8.4	0	0	1.0†	4.5	0	3.7	0	8.2	0	0

* Clumps of growth in culture.

† This organism evidently hydrolyzes certain carbohydrates, splitting off invert sugar faster than it consumes it.

TABLE XIV

AMOUNTS OF CARBOHYDRATE CONSUMED BY CERTAIN MICRO-ORGANISMS

Test substance	Mgms. of glucose											
	B. coli	B. vulgatus	A yeast	Bacillus atrosepticus								
				201	198	197	196	195	187B.1	183.2	180.2	160.1
Glucose	3.5	4.0	9.4	1.1	1.1	0.9	1.7	0.8	1.2	1.2	1.3	1.1
Fructose	3.2	4.4	9.4	1.2	1.3	1.2	1.1	1.2	0.9	1.6	1.5	1.1
Galactose	3.7	1.5	0	1.2	1.2	1.3	1.1	1.5	1.0	1.1	1.5	0.6
Sucrose	0	2.3	0	1.3	1.3	1.1	3.7*	3.6*	1.0	1.4	2.2	1.0
Lactose	2.7	0.1	0	1.9	1.6	2.0	1.2	1.5	1.2	2.1	2.1	1.7
Maltose	3.2	3.4	0.2	0.8	1.8	0.8	1.4	0.4	0.7	0.8	1.2	0.6
Dextrin	1.4	3.3	0	0	0.2	0	0	0	0.2	0	0	0
Potato starch	2.1	3.7	0	0.1	0	0	0	0	0	0	0	0

* Probably too high. See pages 55, 62.

196. *Bacillus coli* and *B. vulgatus* were the only organisms investigated that attacked and used dextrin and starch. The latter, however, appears to have been able to break down both of these substances more rapidly than it utilized the derived sugars (table XIII).

In respect to the amounts of carbohydrates actually utilized, it will be noted upon reference to table XIV that with the several microorganisms under observation (excepting the yeast) the amount is relatively small as compared with the total amount supplied (10 mgms. per cc.). The single instance of *Bacillus vulgatus* on fructose is the only one where the actual amount consumed approaches 50 per cent of the total amount of the sugar provided. The different strains of *Bacillus atrosepticus* consumed only about 10 to 20 per cent of the available supply of carbohydrates. *Bacillus coli* utilized about 30 per cent, and *B. vulgatus* about 40 per cent of the supply. In the light of these findings it would appear that the usual recommendation relative to the amount of carbohydrate that should be supplied in a nutrient medium was a most liberal one. *Bacillus coli* and *B. vulgatus* are usually thought of as being particularly active in fermenting certain saccharides, especially when compared to most plant pathogens. As it is possible that there are numerous bacterial species for which the optimum sugar concentration is 1 per cent or even more, it is doubtful if a less liberal recommendation should be made until the whole matter has been more extensively investigated. However, in the light of these findings, as well as those of Besson, Ranque and Senez ('19), Chambers ('20), Wolf and Foster ('21), and others, it appears that *B. coli,* also some plant pathogenic bacteria, require a less amount of sugar than 1 per cent for optimum development. Besides, there is abundant evidence which indicates that most plant pathogenic bacteria require a very much lower sugar concentration than 1 per cent (cf. also sugar consumption by *B. atrosepticus,* table XIV).

Further evidence of the similarity of the several strains of the blackleg bacillus employed throughout these investigations is brought out in tables X–XIII.

General Summary

I

The blackleg disease of Irish potatoes in North America and Europe is caused by a Schizomycete which should bear the name *Bacillus atrosepticus* van Hall.

The following names are to be considered only as synonyms: *Bacillus phytophthorus* Appel, *B. solanisaprus* Harrison, *B. melanogenes* Pethybridge & Murphy.

The index number "5312-32120-2111" very briefly describes *Bacillus atrosepticus* van Hall, the number being based on the results of the writer's comparative studies.[1] A revised description of this organism appears on pages 43–44.

The blackleg parasite grows best at about 26° C. It withstands extremely low temperatures (–28.2°C.) for several hours.

This organism produces acid and a small volume of gas from each of a number of sugars. The gas-producing capacity is relatively weak, but this capacity can be built up to a certain extent by constant cultivation in the presence of the sugars which it is able to utilize.

The pathogen infects the vines and the tubers of the potato.

Virulence of the parasite, as tested by artificial inoculation, appears to be dependent upon a rather delicate balance of temperature and water relations, and upon the sugar content of the tissues inoculated.

The "incubation period" varied in the experiments under observation and appears to be influenced by the same factors mentioned above. No definite "turning point" was observed to occur, as in the case of many animal diseases.

The above conclusions are based on studies of 12 strains of the potato blackleg parasite, including the 4 "species" originally described as being the cause of the disease.

The strains studied were observed to be morphologically similar. That they were very much alike in their cultural characteristics and physiology was abundantly proven by extensive comparative studies (see data on p. 41).

[1]See Descriptive Chart, Society of American Bacteriologists.

II

Quantitative determinations of carbohydrate utilization show that *Bacillus atrosepticus* cannot hydrolyze potato starch or dextrin.

This organism, however, can utilize the saccharides, glucose, fructose, galactose, sucrose, lactose, and maltose.

The several strains (9) of *B. atrosepticus* investigated were similar in respect to their ability to hydrolyze and utilize the saccharides presented.

Bacillus coli can utilize the carbohydrates, lactose, maltose, dextrin, and potato starch, as well as glucose, fructose, and galactose. The strain investigated did not hydrolyze sucrose, nor was the amount of the original of this sugar reduced.

Bacillus vulgatus can consume the saccharides, sucrose, maltose, dextrin, and potato starch, as well as glucose, fructose, and galactose. The strain investigated could not hydrolyze nor consume lactose.

Under the conditions of the experiments a carbohydrate concentration of 0.25 per cent, 0.4 per cent, and 0.5 per cent would have furnished an ample supply for *Bacillus atrosepticus, B. coli,* and *B. vulgatus,* respectively.

The yeast species investigated utilized only glucose and fructose, of the carbohydrates presented. Both these saccharides were completely removed by this organism in less than 6 days, under the conditions of the experiment.

The saccharides, sucrose, lactose, maltose, dextrin, and potato starch, were not hydrolyzed appreciably if sterilized in the autoclave when dissolved in the Dunham's solution employed.

The disaccharides, sucrose, lactose, and maltose, were hydrolyzed with about equal rapidity in both the autoclave and the steamer when a very small amount of a mineral acid (0.4 per cent normal HCl) was added to "adjust" the Dunham's solution.

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Explanation of Plate

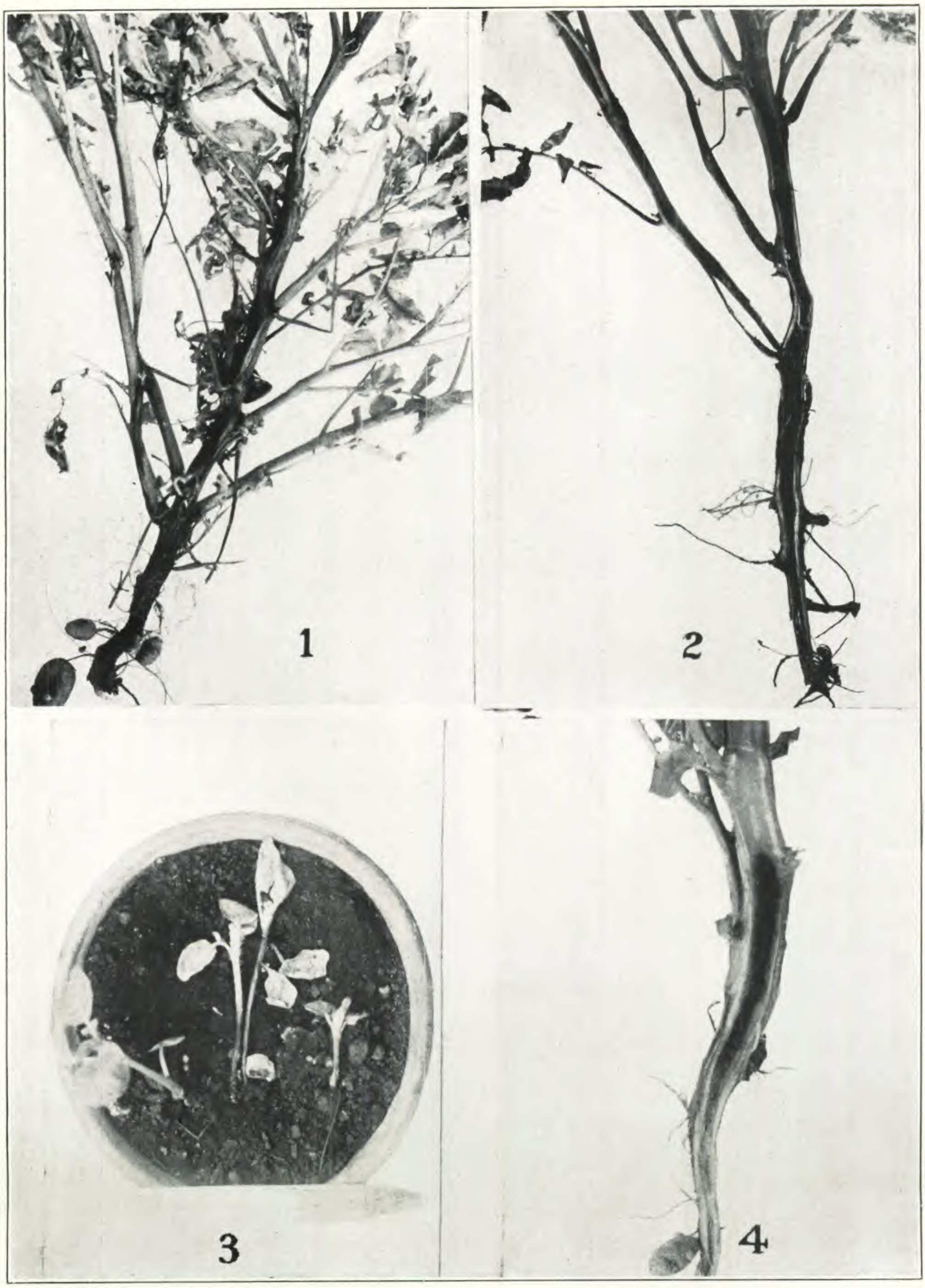
PLATE 1

Fig. 1. Affected plant showing characteristic blackening of stalk and lower branches.

Fig. 2. Similarly affected plant. Lower parts of main stalk and one of the lateral branches cut away to show destruction and blackening of medullary region.

Fig. 3. Young plants prostrated by the disease. Note external signs at base of stalks. The pathogen moved upward into the stalks from the seed piece.

Fig. 4. Median longitudinal section of a recently infected stem showing disease advancing in the pith.



JENNISON—POTATO BLACKLEG

Explanation of Plate

PLATE 2

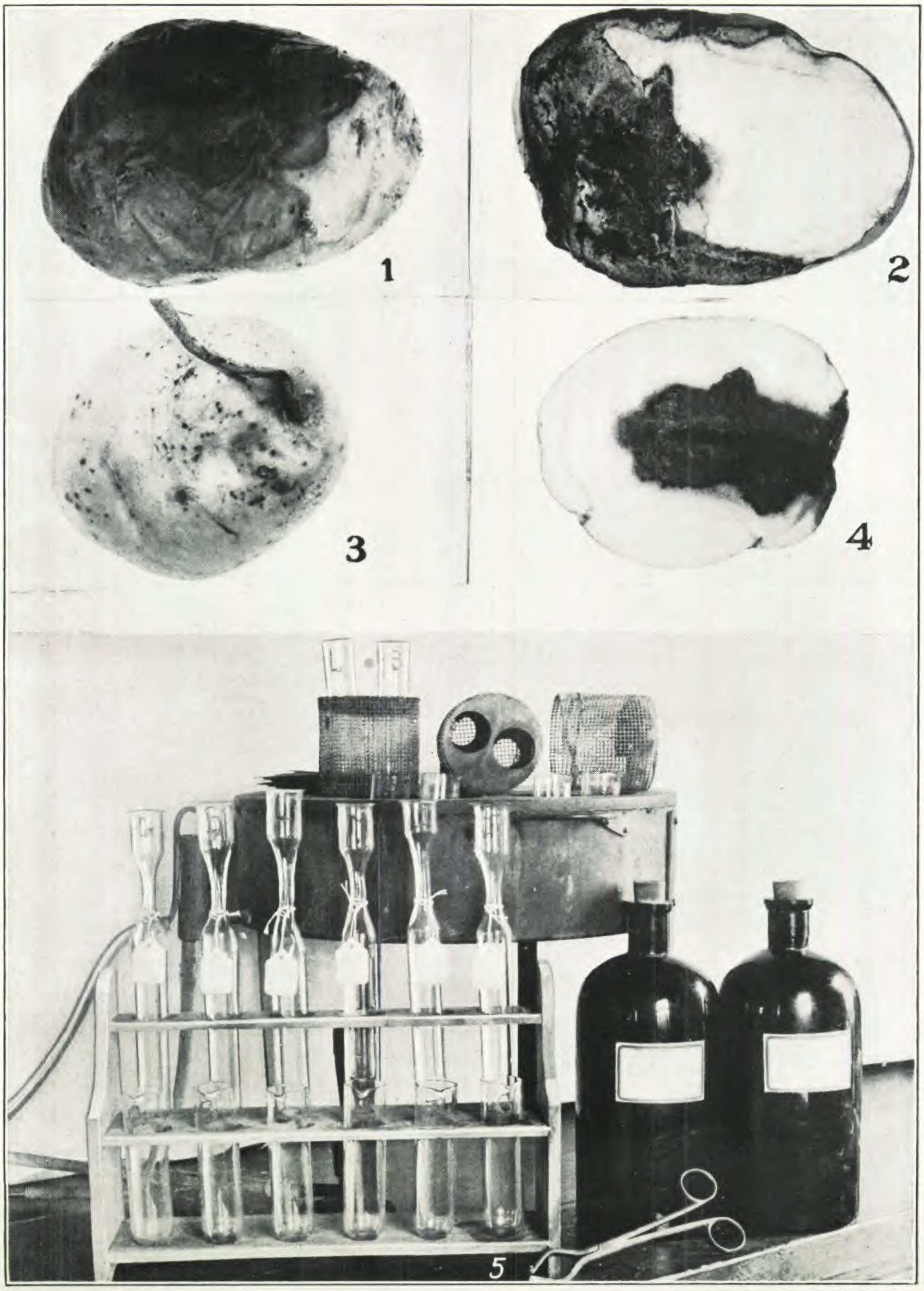
Fig. 1. External view of tuber selected to illustrate characteristics of the rot as well as extent to which it may be manifest externally.

Fig. 2. Internal view of same. The necrosed tissues were quite dry and cheesy.

Fig. 3. External view of affected tuber. The tuber appears to be quite sound except for slight blackening and shrinking of the stolon.

Fig. 4. Internal view of same tuber. Note extent of lesion.

Fig. 5. Apparatus and materials used for determination of sugar consumption.



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